

Coccolithophores

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Coccolithophores

From Molecular Processes
to Global Impact

With 136 Figures, 1 in colour

 Springer

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Preface – Coccolithophore research and the CODENET project.

Coccolithophores are a major component of the oceanic microplankton and of interest to a wide range of scientists. For marine biologists, they are one of the main open ocean primary producers with a distinctive role in the plankton ecosystem. For biogeochemists, they play key roles in the global carbon, carbonate and sulfur cycles and so have important impacts on atmosphere-biosphere-geosphere interactions. For marine geologists, the coccoliths they produce constitute the single most important component of deep-sea oozes and chalks, and provide key floral, isotopic, and biomarker signals for interpreting global change in the geological record. For paleontologists, their exceptional fossil record makes them an outstanding biostratigraphic group and gives them unusual potential for testing evolutionary hypotheses. This grouping of interests was recognized by Peter Westbroek in the early 1990s and formed the basis for a successful multidisciplinary research collaboration focused on the single species *Emiliania huxleyi*. The Global Emiliania Modeling (GEM) initiative including a MAST funded EC project EHUX focused on the role of coccoliths in the global carbon cycle. This stimulated much further research, making coccolithophores in some respects one of the best studied groups of marine microorganisms.

However, advanced research on coccolithophores as biogeochemical agents and paleontological markers has not been paralleled by knowledge of biological diversity. In particular only a very few species of coccolithophores have historically been cultured and even fewer of these studied in detail or used as experimental systems. Typically studies on, for instance, calcification have taken one or two species, usually *Emiliania huxleyi* or *Pleurochrysis carterae* as representatives of coccolithophores as a whole. But are these species really suitable as models for the diverse range of coccolithophores living today and in the geological past? To expand on this small knowledge base we established an EU Training and Mobility of Researchers project CODENET, Coccolithophorid Evolutionary Biodiversity and Ecology Network. In CODENET six key coccolithophore species were selected (see plate on following page) which span the phylogenetic diversity of coccolithophores and which were used as the focus of a wide range of research with the prime aims of: (1) exploring large scale diversity in coccolithophores; (2) elucidating species level variation, through case studies of each species; and (3) advancing our knowledge of coccolithophore ecology and ability to retrieve paleoceanographic data from fossil coccoliths.

The CODENET project ran from 1998 to 2001 involving research teams from across Europe. Through the enthusiastic collaboration of a talented group of post-docs and PhD students the project proved remarkably productive. Specific results were published throughout the duration of project and subsequently (see the CODENET web site for a full bibliography of the ca. 100 project publications www.nhm.ac.uk/hosted_sites/ina/CODENET). There was, however, obvious value in reviewing the work in a larger context. So, we organized a post-project

conference in February 2002 to synthesize results and knowledge gained and to place them in a broader context. With generous support from the Fondazione Monte Verità we were able to hold this meeting in the magnificent setting of the Centro Stefano Frascini, the ETH conference center above Ascona in the southern Swiss Alps. The meeting attracted 50 participants from ten countries, including many contributors from outside the CODENET teams. As on previous occasions, the format of contributions from diverse scientific disciplines focused on a single group of organisms proved very stimulating and all participants found the meeting scientifically informative and intellectually challenging, as well as being very enjoyable socially. The wealth of new material presented and the synergies from combining the different strands of research made it clear that a proceedings volume would be extremely valuable.

The chapters in the book are arranged in thematic groups, but to explain the interrelations between them it is useful to outline the CODENET (*sensu lato*) contributions first. A prime focus of the CODENET research was establishment of new cultures of the target species, followed by immediate use of these cultures to investigate diversity in coccolithophores. This was a somewhat risky strategy, but proved remarkably successful. Multiple isolations were made of all the targeted species, plus many others, and the largest coccolithophore culture collection anywhere was established. Probert & Houdan (p. 217–249) describe this collection and provide a comprehensive review of the art of coccolith culture collection and maintenance. The collection allowed extended investigation of basic aspects of coccolithophore biology as reviewed by Billard & Inouye (p. 1–29). In particular research on life-cycles, combined with spectacular field evidence of life-cycle transitions, resulted in definitive documentation of haplo-diplontic life cycles in coccolithophores and a complete reassessment of the ecological importance of these life-cycles. The culture collection was further used by numerous participants for specialist studies including study of the remarkable pigment diversity in coccolithophores (van Lenning et al., p. 51–73), study of coccolith structure and formation (Young et al., p. 191–216), and studies of coccolith-based geochemical paleoproxies, including stable isotopes, alkenone biomarkers and Sr/Ca ratios (Stoll & Ziveri, p. 529–562).

The extended culture collection also formed the basis for molecular genetic studies, allowing for the first time a detailed molecular phylogeny of coccolithophores to be produced (Saez et al., p. 251–269). Finally, the culture collection was central to our study of species-level variation in coccolithophores. For each of the six selected taxa, we tested species concepts through a combination of physiological culture studies, molecular genetics, morphological analyses of cultured coccoliths, natural populations, and fossil assemblages. In each case supposedly cosmopolitan taxa were shown to be groups of pseudo-cryptic sibling species with narrower ecological tolerances. Geisen et al. (p. 327–366) reviews this work, Quinn et al. (p. 299–326) detail the results for the most intensively studied species, *Calcidiscus leptoporus*, whilst de Vargas et al. (p. 271–298) compare the patterns shown by coccolithophores and planktic foraminifera and discuss evolutionary implications.

A number of additional studies on coccolithophore taxonomy, biogeography, sediment fluxes and geological record were integrated into the CODENET project to complement the culture based work. Ziverj et al. (p. 403–428) present a synthesis of knowledge of the biogeography of the CODENET species and highlight the major potential of new research in this area following the documentation of pseudo-cryptic species. Baumann et al. (p. 367–402) describe detailed work on the magnitude and variability of coccolith fluxes, which is the essential data to allow modeling of their biogeochemical impact. Thierstein et al. (p. 455–479) bridge the gap between biological and geological studies through a comparison of patterns and processes identifiable in plankton communities on short-term ecological and long-term evolutionary timescales. Bown et al. (p. 481–508) continue and document this theme through analysis of changes in coccolithophore diversity through geological time in relation to global change.

To integrate these results into the broader spectrum of coccolithophore research and related fields, we invited leading researchers from outside the CODENET partnership to contribute to the conference and to this volume. These papers are interleaved with the CODENET contributions in this volume. In particular calcification by coccolithophores is reviewed at scales from biochemical processes to geobiochemical impact. Brownlee & Taylor (p. 31–49) review the cell biology of calcification detailing recent elegant work, discuss the continued uncertainties in calcium and carbon pathways and highlight the potential for genomic research to solve such problems. Three contributions address, from different perspectives, the regulation, function and impact of coccolithophore calcification on ecological scales. Tyrell and Merico (p. 75–97) look at the key phenomenon of coccolithophore blooms; Rost and Riebesell (p. 99–125) focus on coccolithophores as components of the carbon cycle and their contribution and response to changes in atmospheric CO₂ levels; Balch (p. 165–189) approaches the same issues from the perspective of coccolithophore physiology and reviews new field approaches to analyze coccolithophore ecology. Hay (p. 509–528) moves analysis of the impact of coccolithophore calcification to geological timescales using synoptic data on distribution of rock types to document the very long term impact of coccolithophores on the global carbon cycle.

Whilst calcification is the prime geochemical impact of coccolithophores, these plankton also have other roles in global change, notably through dimethyl sulfide production as reviewed by Malin and Steinke (p. 127–164). Finally Falkowski et al. (p. 429–453) view coccolithophore evolution and ecological impact in a physiological context, looking at the profound changes in algal pigment groups that have occurred through the Mesozoic and Cenozoic and examining the possible causes and consequences of these changes. Global change on all time scales is increasingly the prime focus of natural environment research and we hope that this volume will provide a valuable source of reference for scientists interested in the role of coccolithophores as well as a stimulus to coccolithophore specialists.

We are maintaining the original conference web site www.coccoco.ethz.ch as a resource for supplementary materials to the individual contributions of this book. Such materials are mentioned in the individual chapters and include (a) pdf files of

articles not yet published at the time of final submission of manuscripts, which will be replaced by appropriate references when these articles are published, (b) supporting data tables, (c) color illustrations for a few figures and graphs printed only in black and white in this book. All of these are mentioned at the appropriate places in the individual articles of the book. The web site also has additional information about the conference held at Monte Verità in 2002 and links to relevant web sites elsewhere.

Hans R. Thierstein

Jeremy R. Young, February 2004

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What is new in coccolithophore biology?

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Summary

Knowledge of the biology of coccolithophores has progressed considerably in recent years thanks to culture studies and meticulous observations of coccospheres in wild samples. It has been confirmed that holococcolithophores and other "anomalous" coccolithophores are not autonomous but stages in the life cycle of oceanic heterococcolithophores. The existence of such heteromorphic life cycles linking former "species" has far reaching consequences on the taxonomy and nomenclature of coccolithophores and should foster research on the environmental factors triggering phase changes. The cytological characteristics of coccolithophores are reviewed in detail with special attention to the cell covering, coccolithogenesis and the specificity of appendages in this group. There have been comparatively few recent studies concerning the cytology of oceanic representatives. Important issues such as status of aplastidic groups, mode of synthesis of holococcoliths/nannoliths and details of the flagellar apparatus need to be addressed. Such morphological data will enable a more natural classification of modern coccolithophores in a phylogenetic perspective.

Introduction

Coccolithophores include all haptophyte algae possessing calcified scales (coccoliths) at some stage in their life cycle. Following the taxonomic revision of the division Haptophyta recently proposed by Edvardsen et al. (2000), coccolithophores belong to the class Prymnesiophyceae which also features non-calcifying organisms.

The biology of extant coccolithophores has been the subject of excellent previous reviews: Pienaar (1994), and relevant chapters in Green and Leadbeater (1994) where coccolithophores were often taken as examples of the haptophytes (see also Inouye 1997). A recent monograph focuses on the model coccolitho-

phore *Emiliana huxleyi* (Paasche 2001). Of the approximately 300 haptophytes in modern oceans, about 200 are in fact coccolithophores and these contribute significantly to the biodiversity of the group (Jordan and Chamberlain 1997). However, as a consequence of recent multidisciplinary research projects such as CODENET (1998–2001), with better sampling/preservation methods and focus on culturing coccolithophores, the number of biological species recognized as authentic is currently in a state of flux. Indeed, one of the more challenging aspects concerning the biology of these organisms is the fact that holococcolithophores and other "anomalous" coccolithophores are not autonomous but stages in the life cycle of heterococcolith-covered oceanic species. In this presentation, current knowledge relative to the different types of heteromorphic life cycles present in coccolithophores and their implications for the taxonomy of the group as a whole will be emphasized. Whereas such heteromorphic life cycles linking two (or more!) former "species" consequently reduce the number of valid species, conversely recent studies demonstrate the existence of fine scale speciation in certain well established taxa (see Sáez et al. 2003; Geisen et al. this volume). The complexity of the existing taxonomy has not hampered the description of new species, whether heterococcolithophores (e.g. Kleijne et al. 2001) or holococcolithophores (e.g. Sym and Kawachi 2000) and a clade of prymnesiophytes of unknown morphology (based on clone library samples only) may represent a new group of coccolithophores (see Sáez et al. this volume). Phylogenetic reconstructions of the Haptophyta are also an area in progress; this should result in a better understanding of the systematics of coccolithophores as well as their status relative to the non-calcifying members of the division (Sáez et al. this volume). The major taxonomic groups within the Prymnesiophyceae featuring extant coccolithophores are listed in Table 1.

In contrast, since the earlier works mentioned in the reviews above, there have been very few studies devoted to the fine structure of coccolithophores. Such studies usually reflect the availability of cultured material and, notably, most recent works are concerned with the description of coastal species, and among these *Pleurochrysis* is still a notorious model organism. Blooms of *P. roscoffensis*, shown to be moderately toxic to brine shrimp, have recently been recorded in saline inland waters (Reifel et al. 2001), which goes to show that such coastal species are more important in the environment than generally thought. However, a number of oceanic species have now been brought into culture (see Probert and Houdan this volume) and this available resource will foster new investigations in coccolithophore research as a whole. For general haptophyte terminology relevant to extant coccolithophores, the reader is referred to the glossary of Jordan et al. (1995).

Table 1. Major taxonomic groups within the Haptophyta featuring heterococcolithophores and place of extant genera mentioned in the text. Based on Young and Bown (1997), Edvardsen et al. (2000) and Kleijne et al. (2001).

DIVISION:	Haptophyta
CLASS:	Prymnesiophyceae
Order:	Isochrysidales
	Family Noelaerhabdaceae (<i>Emiliania</i> [°] , <i>Gephyrocapsa</i> [°])
	Zygodiscales
	Family Helicosphaeraceae (<i>Helicosphaera</i> [*])
	Family Pontosphaeraceae
	Syracosphaerales
	Family Calciosoleniaceae
	Family Syracosphaeraceae (<i>Coronosphaera</i> [*] , <i>Syracosphaera</i> [*])
	Family Rhabdosphaeraceae (<i>Acanthoica</i> [*] , <i>Algirosphaera</i> [*])
	Family incertae sedis (<i>Alisphaera</i> ^{**} , <i>Canistrolithus</i> ^{**})
	Coccolithales
	Family Coccolithaceae (<i>Coccolithus</i> [*] , <i>Cruciplacolithus</i>)
	Family Calcidiscaceae (<i>Calcidiscus</i> [*] , <i>Umbilicosphaera</i>)
	Family Pleurochrysidaceae (<i>Pleurochrysis</i> [°])
	Family Hymenomonadaceae (<i>Hymenomonas</i> [°] , <i>Ochrosphaera</i> [°] , <i>Jomolithus</i>)
	Family Papposphaeraceae (<i>Pappomonas</i> [*] , <i>Papposphaera</i> [*] , <i>Wiggamama</i> [*])
	Family Ceratolithaceae (<i>Ceratolithus</i> ^{***})
	Order incertae sedis
	Family Braarudosphaeraceae (<i>Braarudosphaera</i>)

[°] alternate stage non-calcifying

^{*} alternate stage with holococcoliths

^{**} alternate stage with aragonitic coccoliths

^{***} alternate stage with nannoliths

Cytological aspects

Coccolithophores generally occur as single cells and their typical features have been previously compiled in earlier reviews (Pienaar 1994; Inouye 1997) and are summarized in Fig. 1. Most of the available information is provided from investigations relative to heterococcolithophores since only two holococcolithophores have been sectioned for detailed ultrastructural studies (Klaveness 1973; Sym and Kawachi 2000). The relevant cytological characteristics of coccolithophores are described in the following sections, with emphasis on new findings.

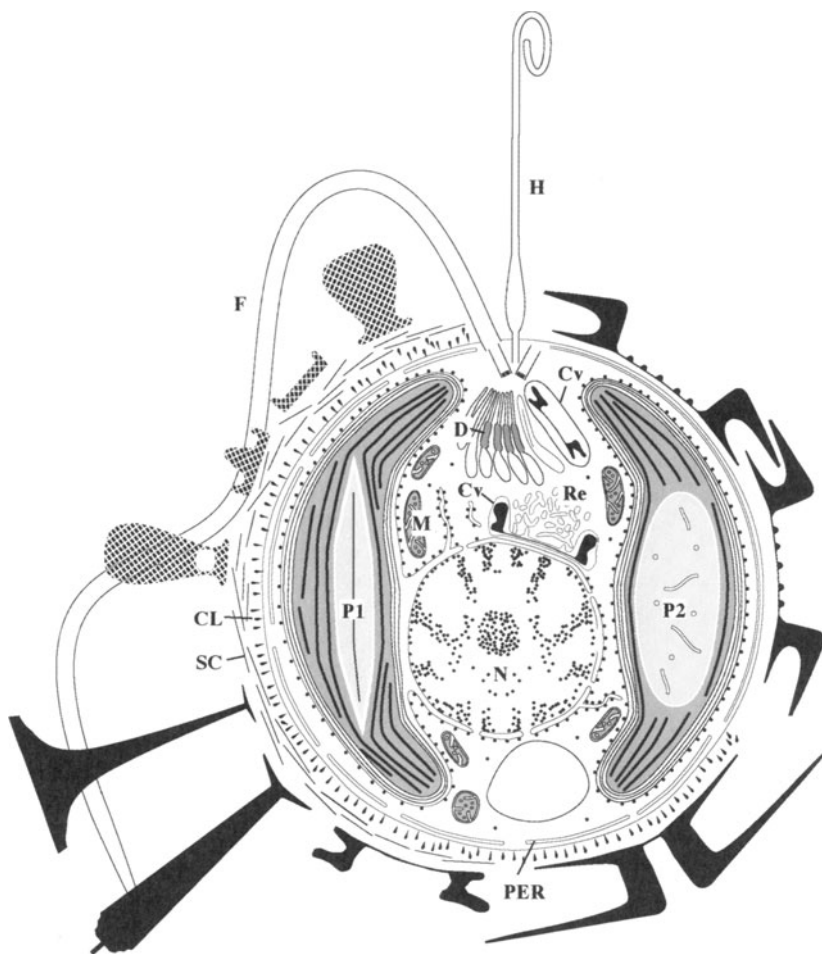


Fig. 1. Diagrammatic representation of cell structures of coccolithophores. Morphological features seen in various coccolithophores are combined in a single figure. Various types of coccoliths are drawn as silhouettes. Two types of coccolith-forming vesicles found in *Pleurochrysis* (top) and *Emiliania* (bottom) are illustrated. Pyrenoid (P1) is typical in the coccolithophores and pyrenoid (P2) is seen in *Emiliania* and *Gephyrocapsa*. Heterococcoliths are blotted black and holococcoliths are blotted by a lattice pattern. *Abbreviations* CL: columnar deposit, Cv: coccolith forming vesicle, D: peculiar dilation of Golgi body, F: flagellum, H: haptonema, M: mitochondrial profiles, N: nucleus, P1: pyrenoid traversed by thylakoids, P2: pyrenoid traversed by tubular structures, PER: peripheral endoplasmic reticulum, Re: reticular body, SC: unmineralized organic scales.

Cell covering

As in other members of the Prymnesiophyceae, the cell wall (periplast) in coccolithophores basically consists of various layers of organic scales held in place by fibrillar or columnar material with presumably adhesive properties (Fig. 1). The unit of cell covering in prymnesiophytes is considered to be a two-layered microfibrillar scale (Leadbeater 1994). Coccolithophores are distinctive in that the distal scales of the periplast are generally calcified, termed coccoliths, and visible with the light microscope. Cell coverings (including chemical composition) in prymnesiophytes or in coccolithophores have previously been extensively reviewed by Leadbeater (1994) and Pienaar (1994) respectively. Here we present aspects of cell coverings which are relevant to life cycles and systematics.

Organic body scales

Body scales in coccolithophores designate the layers of non-calcified scales located closest to the plasmalemma and produced by the Golgi apparatus. The majority of coccolithophores (including holococcolithophores) examined possess such scales and these represent the only type of cell wall in certain families where the haploid stage of the life cycle is non-calcifying: Noelaerhabdaceae (*Emiliania*; *Gephyrocapsa*, Probert unpublished results), Pleurochrysidaceae (*Pleurochrysis*), Hymenomonadaceae (*Hymenomonas* (Fresnel 1994); *Ochrosphaera* (Fresnel and Probert in press)). Notable exceptions are *Emiliania huxleyi* and *Umbilicosphaera foliosa* (= *U. sibogae* var. *foliosa*) where the heterococcolith-covered stages lack an underlayer of body scales (see references in Pienaar 1994).

Relative sizes, shape and ornamentations of organic body scales are variable and such aspects must be carefully observed in shadowcast whole mounts or thin-sections, and viewed with the transmission electron microscope (TEM). Reduced body scales, whatever their shape or patterning, are sometimes observed at the flagellar pole of certain motile stages of coccolithophores and have been termed haptonematal scales. While they may be related to the presence of a haptonema (e.g. *Crystallolithus*-stage of *Coccolithus*), in some instances they have been reported despite the absence of an emergent haptonema. Such is the case in the Hymenomonadaceae where the haptonema is vestigial: haptonematal scales are remnant in members of the genera *Hymenomonas* and *Jomonolithus*, while they are altogether absent in *Ochrosphaera* (Fresnel and Probert in press). Remnant haptonematal scales could therefore indicate recent secondary loss of the haptonema.

Two main types of body scales may distinguished differing in their general shape and ornamentations: (1) circular body scales, generally rimmed, with apparently identical ornamentations on both sides, i.e. patterned with concentric plus radial fibrils; in favorable cases however, when thick scales are present, the two-layered organization typical of prymnesiophyte scales is recognizable, the concentric pattern showing on the distal face, the radiating fibrils on the proximal face, as in *Coccolithus* (see recent observations by Houdan et al. in press) and also in *Cruciplacolithus* (see Fresnel 1986, although interpretation of the two sides of

the body scales was different); (2) elliptical, rimless body scales with distinctly different ornamentations on each face (distal pattern of concentric fibrils; proximal pattern of radiating fibrils arranged in four quadrants). Billard (1994) argued that this heteromorphism in body scales might be indicative of ploidy levels in the biphasic life cycle, type (1) scales being found in heterococcolithophores (presumably the diploid generation) and type (2) scales in holococcolithophores or non-calcifying stages (presumably alternate haploid generations).

The haploid, unmineralized motile stage of *Emiliana* features a third type of body scale (Klaveness 1972; Green et al. 1996) which is also present in motile stages of *Gephyrocapsa* (Probert unpublished results): type (3) scales are variable in shape, circular to elliptical, and monomorphic (with a simple pattern of radiating fibrils arranged in four segments which do not meet at a common point at the center of the scale). These thin scales, which are smaller than types (1) or (2), are considered single-layered (Green et al. 1996). Identical body scales are present in the non-mineralized genus *Isochrysis* (Billard and Gayral 1972; Green and Pienaar 1977), and are so far distinctive of the order Isochrysidales to which the Noelaerhabdaceae belong (Edvardsen et al. 2000).

Another type of body scale has been recently observed in the heterococcolithophore *Algirosphaera robusta*, a member of the Rhabdosphaeraceae: these minute scales (half the size of the scales of *Emiliana*) are strongly elliptical, rimmed, and bear a pattern of radiating fibrils arranged in four quadrants with a well defined elongated central ridge (Probert et al. in press). They may represent a novel type of apparently monomorphic body scales, which so far has not been reported in any other heterococcolithophore.

Coccoliths

Coccolithophores produce two main types of coccoliths which differ in their morphology: heterococcoliths, formed of crystal-units of variable sizes and shapes and holococcoliths, made of a single type of minute crystallites (see Young et al. this volume). Within these basic categories, various terms are used by workers on living coccolithophores and based on morphological characteristics of certain taxa: cricoliths, helicoliths, pappoliths, etc. for heterococcoliths; calyptroliths, crystalloliths, laminoliths, etc. for holococcoliths (see Young et al. 1997 for terminology). The abundance of such terms reflects the high diversity of coccolith morphology. The possible biological functions of coccoliths have been comprehensively reviewed by Bown and Young (1998).

Two families of extant coccolithophores produce so-called nannoliths (Ceratalithaceae, Braarudosphaeraceae) which are anomalous calcareous structures lacking the typical features of hetero- or holococcoliths. Whereas the Ceratalithaceae have heterococcolithophore stages in their life cycle, the affinities of the Braarudosphaeraceae are still uncertain, although living cells have golden brown plastids, and motile cells (with two equal flagella and no haptonema) have been reported once in *Braarudosphaera magnei* (Lefort 1972). *Polycrater galapagensis* produces unusual aragonitic coccoliths (often considered as nannoliths) and its assignment to the haptophytes was tentative until it was shown to possess a hap-

tonema (Thomsen and Buck 1998). Furthermore, various "species" of *Polycrater* have recently been shown by Cros et al. (2000a) to be in fact stages in the life cycle of heterococcolithophores. It is therefore becoming increasingly clear that coccolithophores produce different types of coccoliths/nannoliths during their life cycle, each generation being characterized by the presence of a distinct type of calcareous structure or by the absence of calcified structures altogether (see Table 1).

Coccoliths may be of similar size or morphology on a single cell, or there may be several distinct coccolith forms and sizes (e.g. circum-flagellar coccoliths). In most cases coccoliths (which may be interlocking or not) form a single layer external to the body scales, but species of the large genus *Syracosphaera* consistently exhibit dithecatism, i.e. two discrete layers of heterococcoliths of different types (e.g. Cros 2000). A few coccolithophores over-produce heterococcoliths of a single type, and the coccosphere may thus become multilayered (*Emiliania*, see Paasche 2001; *Cruciplacolithus*, Fresnel 1986). A specific process has recently been described in cultures of the coastal species *Ochrosphaera neapolitana*: coccolith formation does not continue once the coccosphere is complete and the heterococcoliths (tremaliths) progressively undergo extracellular over-calcification which is thought to occur by continued growth of existing coccolith crystals; ultimately individual coccoliths are no longer distinguishable and the resulting over-calcified cell (pseudo-cyst) is thought to represent a resistant stage (Fresnel and Probert in press).

The covering of holococcoliths may be enclosed within a continuous investment termed "skin" or envelope, as in the *Crystallolithus*-stage of *Coccolithus* or in *Calyptrorphaera sphaeroidea*. This envelope has a fibrillar microarchitecture in the former case (Rowson and Leadbeater 1986) and is interpreted by Sym and Kawachi (2000) as being composed of cohesively packed organic scales. This external envelope is lacking in *Calyptrorphaera radiata* (Sym and Kawachi 2000).

Coccoliths are typically based on an organic baseplate scale with microfibrillar components of prymnesiophyte scales. Size, thickness and patterning of the baseplate scale is highly variable among species; in *Syracosphaera pulchra*, the proximal coccoliths have baseplates whereas distal coccoliths do not (see Pienaar 1994). Mature coccoliths of *Emiliania* and *Gephyrocapsa* lack microfibrillar baseplates but a thin layer of polysaccharide material (analogous to an organic baseplate) is present in the coccolith making vesicle of *Emiliania* (see references in Paasche 2001).

Haptonema and flagellar apparatus

In several articles published in the 1990's, the haptonema and flagellar apparatus of the Haptophyta were reviewed in detail (Green and Hori 1994; Inouye and Kawachi 1994; Pienaar 1994). Since then, most studies have been conducted on non-coccolith-bearing members of the Prymnesiophyceae, especially the genus *Chrysochromulina* (Birkhead and Pienaar 1994a, 1995; Eikrem and Throndsen 1998; Eikrem and Moestrup 1998; Eikrem and Edvardsen 1999; Jensen and

Moestrup 1999), and very few investigations have been undertaken on the coccolithophores (Sym and Kawachi 2000). However, these works, in combination with previously published data, have demonstrated both great consistency in certain respects and variations of the haptonema and the flagellar apparatus within the Prymnesiophyceae and the coccolithophores.

Haptonema

The haptonema is a multi-functional organelle unique to the haptophytes (Fig. 1). It adheres to substrata, coils and uncoils. It is responsible for prey capture in some members of *Chrysochromulina* (Kawachi et al. 1991; Jones et al. 1993; Kawachi and Inouye 1995). It is typically comprised of six or seven microtubules surrounded in part or entirely by the haptonematal endoplasmic reticulum (ER) which is an extension of the peripheral endoplasmic reticulum (Fig. 2A). The haptonematal microtubules increase in number up to eight or nine toward the base, changing configuration from a ring in the free part, an arc around the insertion region, to two rows of four microtubules or a diamond pattern at the most proximal end. The transition region from the haptonema base to the emergent part is a complex structure comprised of a tongue-like extension toward the arc of microtubules and electron dense material in which microtubules are embedded (Fig. 2A). All these features seem to be common in the Prymnesiophyceae, although the number of microtubules and their configuration is different in reduced forms.

Although the emergent haptonema is known in various orders and families of coccolithophores, ultrastructural studies have been conducted for few genera. The number of microtubules in the emergent part is six in the holococcolith-bearing motile phase of *Coccolithus* (Manton and Leedale 1963) and *Calyptrorphaera sphaeroidea* (Klaveness 1973). It is seven in the emergent part and eight at the base in *Syracosphaera pulchra* (Inouye and Pienaar 1988) while *Algirosphaera robusta* has the same number of microtubules in the base, but one less (six) in the emergent part of the haptonema (Probert et al. in press). *Helicosphaera carteri* (Helicosphaeraceae) also has a haptonema comprised of six microtubules in the free part and eight at the base (unpublished observation). The haptonema of these coccolithophores usually does not coil under normal conditions, but only when cells are exposed to stress such as desiccation or when cells are dead. Rapid coiling as seen in *Chrysochromulina* has never been reported in the coccolithophores. The utilization of the haptonema to adhere to substrata is also not so conspicuous in these coccolithophores. The emergent haptonema of coccolithophores, when present, seems to be morphologically similar to that of other members of the Prymnesiophyceae (e.g. *Chrysochromulina*), but less functional and appears to act mainly as an obstacle-sensing device. When cells sense obstacles with the tip of the haptonema, rapid backward swimming occurs as an avoidance response.

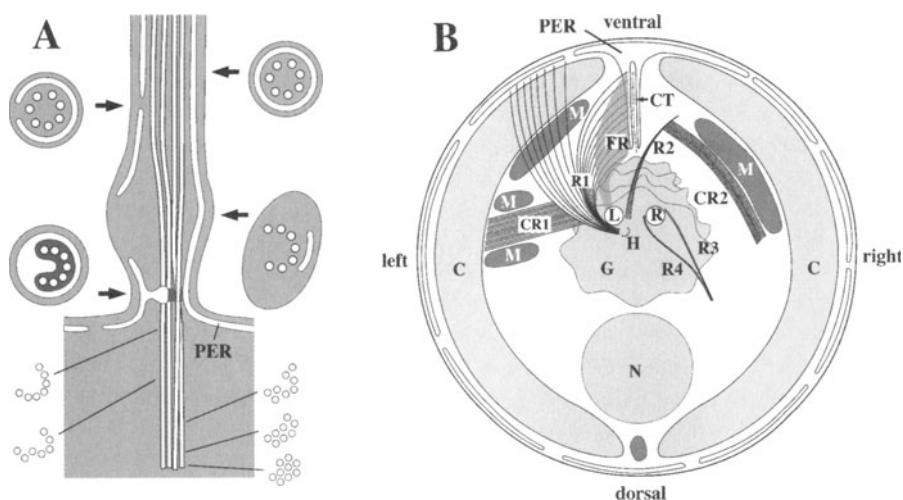


Fig. 2. **A.** Schematic representation of a haptonema showing configuration of microtubules and endoplasmic reticulum. **B.** Diagrammatic representation of a cell viewed from the apex, showing typical configuration of flagellar apparatus components and other organelles.

Abbreviations C: chloroplast, CR1: crystalline root arising from R1, CR2: crystalline root arising from R2, CT: cytoplasmic tongue, FR: fibrous root, G: Golgi body, H: haptonema, M: mitochondrial profiles, L: left basal body, N: nucleus, PER: peripheral endoplasmic reticulum, R: right basal body, R1: root 1, R2: root 2, R3: root 3, R4: root 4.

The haptonema is often vestigial in various groups of coccolithophores. A reduced bulbous haptonema is known in most species of *Pleurochrysis* (Pleurochrysidaceae) and *Hymenomonas roseola* (Hymenomonadaceae) (Manton and Peterfi 1969). In an unnamed species of *Pleurochrysis*, six microtubules are arranged more or less in an arc in the bulbous region and haptonematal endoplasmic reticulum is also arranged in an arc (Inouye and Pienaar 1985). In *H. roseola*, small vesicles occupy the bulbous region. The haptonema base of *Pleurochrysis* seems to be constant (two rows of four microtubules) (Gayral and Fresnel 1983; Inouye and Pienaar 1985; Fresnel and Billard 1991). In many taxa of the Hymenomonadaceae, the haptonema is reduced to the haptonematal base, and the number of microtubules are variable depending on taxa (three in *H. lacuna*, five in *H. coronata* arranged in a U-shape throughout its length, at least five in *H. globosa*, five in *Ochrosphaera neapolitana* and five in *Jomonolithus littoralis*) (Gayral and Fresnel-Morange 1971; Pienaar 1976; Gayral and Fresnel 1976; Inouye and Chihara 1988; Roberts and Mills 1992). The flagellate cells of *Emiliania* and *Gephyrocapsa* (Noelaerhabdaceae) lack a haptonema, but their non-coccolith bearing relative, *Isochrysis*, possesses a very short rudimentary haptonema that is comprised of five microtubules in the free part and at the base (Hori and Green 1991). *Crucioplacolithus neohelis* is the only taxon in the Coccolithaceae of which the haptonematal

base has been described in detail (Fresnel 1986; Kawachi and Inouye 1994). The flagellate cells of *C. neohelis* have five microtubules at the base. In the Calcidiscaceae, a haptonematal base consisting of eight microtubules (two rows of four microtubules) has been described in non-motile cells of *Umbilicosphaera foliosa* (Inouye and Pienaar 1984). These tendencies to reduction and less effective functions of the haptonema suggest that the haptonema seems to be a less important organelle in the biology of certain coccolithophores if compared with other prymnesiophytes.

In contrast, the haptonema is remarkable by its length and seemingly coiling abilities in members of the aplastidic Papposphaeraceae (e.g. Thomsen et al. 1998; Thomsen and Buck 1998) and could possibly be involved in prey capture. None of these tiny coccolithophores have been grown in culture, however, and available observations are based exclusively on transmission electron micrographs, except for *Balaniger balticus* (the alternate stage of *Pappomonas virgulosa*, see Østergaard 1993) where the haptonema was seen to coil in living cells (Thomsen pers. com.).

Flagellar apparatus

The flagellar apparatus is a complex structure involved in various cellular functions, such as mitosis and cytoskeleton formation, and its morphological features are believed to be evolutionarily conservative. Analysis of flagellar structure is therefore informative in taxonomy and phylogenetic analysis. In the Haptophyta, the flagellar apparatus is unique and complex, due to the involvement of the haptonema. Two flagellar basal bodies and the haptonematal base are arranged in an absolute configuration. The haptonema is positioned close to the left basal body, and the concave side of the C- or U-shaped array of microtubules is always oriented to the left basal body (Fig. 2B). This basal body is designated as mature in terms of the generation of basal bodies and flagella, and it is seen as the longer flagellum under the light microscope (Beech et al. 1988). The right basal body is situated at a distance from the haptonematal base and corresponds to the shorter flagellum. The latter is the immature basal body (flagellum), and is destined to become the mature basal body in the next generation. This configuration seems to be universal in the Haptophyta. The two classes of the Haptophyta, Pavlovophyceae and Prymnesiophyceae, are however distinct in other aspects of the architecture of the flagellar apparatus (see Green and Hori 1994 for the Pavlovophyceae).

In the coccolithophores, the flagellar apparatus has been studied in detail mainly for members of the Pleurochrysidaceae and Hymenomonadaceae. The flagellar apparatus in these coccolithophores is the most complex among the haptophytes so far investigated, and has been adopted as a standard for comparison within the Prymnesiophyceae. Species in these families possess four microtubular roots, termed R1, R2, R3 and R4 (Fig. 2B). Of these, R1 and R2, both associated with the left basal body, are conspicuous because of the presence of closely packed bundles of microtubules, termed crystalline roots (Beech and Wetherbee 1988). R1 is a large sheet of microtubules, originating in the proximity of the haptonematal base. These microtubules run upward along the flagellar depression,

then, some of these pass over the mitochondrial profile and chloroplast situated at the left side of the cell. Other microtubules extend toward the ventral side of the cell, where they form a complex with the fibrous root originated from the left basal body, and extend into a thin space of cytoplasm delineated by extensions of the peripheral endoplasmic reticulum. This structure is collectively called the cytoplasmic tongue (Beech and Wetherbee 1988). Its functions are not well understood, but it has been suggested that the cytoplasmic tongue is responsible for contraction of the cell (this structure was termed a 'contractile root' by Fresnel and Billard 1991) or related to scale formation due to the proximity to the forming side of the Golgi body (Gayral and Fresnel 1983; Beech and Wetherbee 1988). The cytoplasmic tongue is conspicuous in many flagellate coccolithophores, but it is also found in *Chrysochromulina* sp. (Birkhead and Pienaar 1995). R2 originates from between the basal bodies near the left basal body and beneath the distal connecting fiber, and is comprised of a small number of microtubules (up to seven). Another crystalline root (CR2) is associated with R2 and extends toward the right side of the cell. These two crystalline roots are well developed in most coccolithophores so far investigated, however, some coccolithophores have only one crystalline root or completely lack both. In *Hymenomonas coronata* (Roberts and Mills 1992) and *Calyptrosphaera radiata* (Sym and Kawachi 2000), CR1 is absent though CR2 is well developed in both algae. In contrast, *Cruciaplacolithus neohelis* possesses a CR1, though it is vestigial and comprised of only about five microtubules, and CR2 is missing. In this species, well developed CR1 and CR2 appear in preprophase, suggesting their conversion to a mitotic spindle (Kawachi and Inouye 1994). The crystalline roots are completely absent in *Syracosphaera pulchra* (Inouye and Pienaar 1988) and *Algirosphaera robusta* (Probert et al. in press). The crystalline roots used to be thought to be unique to the coccolithophores (e.g. Kawachi and Inouye 1994). However, structures comparable to the crystalline roots have been found in several non-coccolithophore prymnesiophytes. *Isochrysis galbana* (a non-coccolith-bearing member of the Isochrysidales) has a very complicated R1 root, i.e. it has CR1 (as r1c) and an additional bundle of microtubules arising from CR1 (as r1b) (Hori and Green 1991). Since *Isochrysis* is believed to be a taxon which has secondarily lost the ability of coccolith formation (it is a close relation of *Emiliania* and *Gephyrocapsa*), it is not surprising that *I. galbana* possesses crystalline roots. However, its complex R1 root, together with other features such as the involvement of a reticular body in coccolith formation, suggests that this group has undergone a unique evolution within the coccolithophore lineage. CR2 is missing in *I. galbana*. The crystalline root is also present in some species of *Prymnesium* (Birkhead and Pienaar 1994b), and *Chrysochromulina* (Birkhead and Pienaar 1995; Edvardsen et al. 1996), which may be phylogenetically distant from the coccolithophores. In the latter, R3 and R4 are simple roots compared with R1 and R2. Root R3 originates from the right side of the right basal body and R4 originates from the left side of the right basal body (Fig. 2B). These two roots merge at a distance and this combined root extends towards the dorsal side of the cell. The function of this combined root is not understood but it is suggested in *Chrysochromulina* that it is involved in determining the site on the

cell membrane where scale release takes place (Jensen and Moestrup 1999), which could also be applicable to the coccolithophores.

Various fibrous bands, some of which appear to be consistently present in most taxa, connect the flagellar basal bodies and the haptonematal base. Most typically, the two basal bodies are interconnected by distal, intermediate and proximal striated connecting fibers. The haptonematal base is connected to the two basal bodies by haptonematal fibers. Variations are present in the presence or absence of the intermediate connecting fibers and the haptonematal fibers and their striated or non-striated nature.

Transition region and flagellar axoneme

Unique structures of the flagellar transitional region are known in *Pleurochrysis carterae* (Beech and Wetherbee 1988). Tiers (up to eight) of electron dense rings are situated at the level of the cell membrane and distal to this is a transitional plate called an axosome. In the proximal part of the flagellar axoneme, a helical band is situated between the doublets and central pair of microtubules. Similar structures seem to be widely distributed in the Pleurochrysidaceae (e.g. Henry et al. 1991) and Hymenomonadaceae (e.g. Manton and Peterfi 1969; Gayral and Fresnel 1983; Roberts and Mills 1992). The axosome also seems to be widely distributed in various coccolithophores, although not so elaborated as that of *P. carterae* (e.g. Sym and Kawachi 2000). In the flagellate cells of *Emiliania* and *Gephyrocapsa*, there is a dense band in the basal region of the axoneme (unpublished observation) that is similar to the fibrous plug known their non-coccolith bearing relative *Isochrysis galbana* (Hori and Green 1991). This structure is probably a characteristic of the Noelaerhabdaceae.

In general, most features of the haptonema and flagellar apparatus of the coccolithophores are primitive (plesiomorphic) characters, and consequently, it is difficult to illustrate uniqueness of the coccolithophores by these features.

Chloroplasts

Coccolithophores generally contain two golden brown chloroplasts with chlorophylls *a* + *c*. For further pigment composition, the reader is referred to Van Lenning et al. this volume. Presence of a single chloroplast has been documented only in some holococcolithophores (species of *Calypトロsphaera* available in culture) and confirmed by serial sectioning. In the Haptophyta, the existence of only one chloroplast is typical of the members of the Pavlovophyceae (Edwardsen et al. 2000) and a single plastid is also observed in certain non-calcifying prymnesiophytes such as *Isochrysis* and *Chrysotila* (Isochrysidales).

The chloroplast, which features thylakoids in stacks of three, typically lacks a girdle lamella and is surrounded by four membranes, two of which represent the chloroplast endoplasmic reticulum. The presence of these additional membranes is thought to reflect the secondary origin of haptophyte plastids in the endosymbiotic hypothesis of plastid evolution. The outer membrane of the nuclear envelope is

continuous with the chloroplast endoplasmic reticulum. Chloroplast DNA in coccolithophores, as in other haptophytes, is dispersed within the stroma. The plastid genome size of *Ochrosphaera neapolitana* has been recently investigated and it is significantly larger than in other chlorophyll *a* + *c*-containing algae (Sáez et al. 2001).

Each chloroplast always contains a pyrenoid which is immersed in the majority of the coccolithophores examined so far (Fig. 1), except for members of the families Pleurochrysidaceae and Hymenomonadaceae where it is bulging and located on the internal face of each plastid. In *Ochrosphaera* (Hymenomonadaceae) the bulging pyriform pyrenoids are stalked and particularly conspicuous (Fresnel and Probert in press). These bulging pyrenoids may represent a derived character in certain Coccolithales. Immersed pyrenoids of coccolithophores show some diversity at the ultrastructural level: they are usually traversed by a number of thylakoids, except for the Noelaerhabdaceae where the pyrenoid stroma contains tubular profiles (Fig. 1). The product of photosynthesis in coccolithophores is considered to be a β 1-3 glucan (chrysolaminarin) as in other Prymnesiophyceae.

As a rule, there is no stigma in members of the class Prymnesiophyceae, and coccolithophores are no exception. Despite the absence of an eyespot, motile stages of some coccolithophores examined in culture may be positively phototactic such as the *Crystallolithus*-stage of *Coccolithus* (Houdan pers. com.; see also Fresnel 1994; Fresnel and Probert in press).

Although the majority of coccolithophores are photosynthetic (all cultured species at least), polar representatives of the weakly calcified family Papposphaeraceae (including *Pappomonas*, *Papposphaera*, *Wigwamma* and their alternate stages with holococcoliths; Thomsen et al. 1991; Østergaard 1993) and an allied genus of holococcolithophores (*Ericiulus*) are heterotrophic organisms featuring long, and apparently coiling haptonemata (Marchant and Thomsen 1994; Thomsen et al. 1995). The lack of chloroplasts (and hence absence of any red chlorophyll autofluorescence) was verified using epifluorescence microscopy (Thomsen et al. 1995). The same genera of Papposphaeraceae have now also been found in temperate areas (Thomsen and Buck 1998; Cros and Fortuño 2002) but it is not known whether these small coccolithophores are also aplastidic outside polar regions characterized by prolonged periods of darkness. As Thomsen and Buck (1998) pointed out, thin-sectioning of embedded material and the search for chloroplasts and/or food vacuoles with the TEM is necessary in the Papposphaeraceae to demonstrate mixotrophy and possibly phagotrophy.

Phagotrophy has frequently been documented in non-mineralized haptophytes with plastids: in *Chrysochromulina*, the long and coiling haptonema may play an active part in prey capture (Kawachi et al. 1991), while in *Prymnesium* ingestion is by means of pseudopodial development at the non-flagellar pole with no involvement of the short haptonema (Tillmann 1998). In coccolithophores, information on phagotrophy is lacking except for the holococcolith-covered *Crystallolithus*-stage of *Coccolithus* where ingestion of graphite particles was once recorded (Parke and Adams 1960). Holococcolithophores being alternate motile stages with presumably a less rigid coccolith covering, are in fact likely candidates for phagotrophy. Non-calcifying motile stages of other coccolithophore

families are also potential candidates, whether a haptonema is present or not (e.g. *Emiliania* motile cells; Paasche 2001). The question of active phagotrophy (or mixotrophy) should therefore be addressed in coccolithophores (and particularly in the Papposphaeraceae), in view of its potential ecological significance in the microbial food web.

Golgi apparatus and coccolithogenesis

The single, large and highly polarized dictyosome, with its peculiar dilated central cisternae, is a distinctive attribute of the Prymnesiophyceae (Fig. 1). In coccolithophores it is involved both in the synthesis of organic scales and coccolithogenesis. Sequential synthesis of the organic body scales is a process well documented (see earlier reviews by Leadbeater 1994; Pienaar 1994) and haploid non-calcifying cells of *Pleurochrysis* are still used as models to study transport mechanisms of secretory products across the Golgi body. A recent study (Hawkins and Lee 2001), using improved fixation and staining methods and quantitative morphology analyses, challenges the cisternal-progression model generally assumed for scale formation in this coccolithophore. Their results in *Pleurochrysis* sp. show that body scales form in the trans-Golgi network and that abstricted cisternal fragments of the basal trans-Golgi network develop into discoid scale-bearing prosecretory vesicles. These mature into secretory vesicles prior to exocytosis of the scales to the cell surface. Secretory vesicles may contain up to five scales following sequential fusions of prosecretory vesicles. They distinguish two morphological types of cisternal dilations which are centers of radial microfibril synthesis. Previous evidence in *P. sherffellii* had shown that radial fibrils are laid down before the spiral/concentric fibrils (see Leadbeater 1994). Hawkins and Lee (2001) also describe novel, bottlebrush-shaped macromolecules which are related to the biogenesis of scales and may also account for the "columnar" deposit outside the cell membrane in various coccolithophores. According to these authors, body scale formation in coccolith-covered stages of *Hymenomonas lacuna* (Pienaar 1994) follows the same vesicle shuttle progression model as in *Pleurochrysis*.

Coccolithogenesis has been investigated mostly in cultured heterococcolith-bearing species and it is now becoming apparent that mechanisms may vary according to the morphological type of coccolith produced. Basically, heterococcolith growth is intracellular and occurs inside Golgi vesicles or Golgi-derived compartments and the coccolith is extruded to the cell surface (generally close to the flagellar pole) when fully calcified. Diversity is observed in the manner in which components for the construction of the coccolith are transported to the coccolith vesicle and in the role of the endomembrane system in shaping the forming coccolith (Fresnel and Probert in press).

In *Pleurochrysis*, once synthesis of the organic baseplate is completed inside a Golgi cisterna, the scale is transferred to distal Golgi-derived vesicles where calcite nucleation and growth occur in the presence of densely stained granules termed coccolithosomes. Recent data on coccolith development and its biochemical aspects in *Pleurochrysis* will be found in Marsh (1999). Presence of discrete

coccolithosomes are typical of *Pleurochrysis* species and no particular cellular component is involved in shaping the small coccoliths (cricoliths). In *Ochrosphaera*, the vesicle containing the baseplate also migrates away from the Golgi, and subsequently dilates when close to the peripheral endoplasmic reticulum (PER). Invaginations of the peripheral endoplasmic reticulum form a tubular matrix, containing densely stained material inside the coccolith vesicle. In *Ochrosphaera* the peripheral endoplasmic reticulum and its tubular invaginations are thought to be involved in modeling the shape of the future coccolith (tremalith) which calcifies at a later stage (Fresnel and Probert in press). In *Emiliania*, the coccolith vesicle seems to result from fusion of smaller Golgi derived vesicles. The coccolith vesicle remains tightly apposed to a flattened section of the nucleus and formation of a thin baseplate, lacking a microfibrillar pattern, is the first stage of the developing coccolith. A reticular body (Fig. 1) of anastomosing tubes is added to the vesicle and calcification proceeds. When it is completed, the coccolith vesicle is detached from the nucleus (see Paasche 2001 for recently acquired information relevant to biochemical aspects of coccolith formation). In both *Emiliania* and *Gephyrocapsa* where the coccoliths are probably formed one by one, the role of the nucleus in shaping the early stages of the growing coccolith (placolith) is morphologically very apparent. *Umbilicosphaera foliosa*, which lacks organic body scales, also produces its large coccoliths (placoliths) one at the time and the endoplasmic reticulum system, together with the nucleus and the Golgi body seem to participate in shaping the coccolith vesicle (Inouye and Pienaar 1984).

Formation of holococcoliths is a process not yet fully understood, with only two case studies. In the *Crystallolithus*-stage of *Coccolithus*, the baseplate scales of the crystalloliths are synthesized inside Golgi-associated cisternae, extruded into the periplast, and calcium carbonate deposition is thought to take place extracellularly (Rowson et al. 1986). The envelope or "skin" surrounding the external layers of holococcoliths is suggested to play a role in crystallogenesis, maintaining a favorable environment for precipitation of calcium ions on the distal side of the baseplate. The baseplate scales of *Calyptraosphaera radiata* are produced as in *Crystallolithus*, but Sym and Kawachi (2000) furnish some evidence that the calcite crystals form within scale-containing cisternae of the Golgi. As opposed to *Crystallolithus*, an outer envelope is lacking in *C. radiata*. However, since no mature calyptroliths are found in the dictyosome, crystal assemblage is thought to occur externally. Clearly further investigations are needed to elucidate the system of calcite assembly in holococcoliths.

Life cycles

Coccolithophores reproduce asexually by binary fission and, generally following mitotic division, the coccoliths are redistributed on the daughter cells. In cultures of *Ochrosphaera neapolitana*, the cell divides inside the coccosphere and one of the daughter cells escapes and forms a new covering of coccoliths while the other

conserves the initial coccosphere (Fresnel and Probert in press). When motile and non-motile generations alternate in the life cycle, each is capable of vegetative reproduction as confirmed by culture studies: whether in oceanic forms (e.g. non-motile *Emiliania* and its alternate flagellate scaly stage; non-motile *Coccolithus* and its alternate motile stage *Crystallolithus*) or in littoral forms (e.g. motile *Pleurochrysis* and its alternate benthic scaly stage) (see references in Billard 1994). Benthic non-calcifying stages may also produce flagellate cells, considered as swimmers since they perpetuate the same generation. This is the case in families of coastal coccolithophores (Pleurochrysidaceae, e.g. Fresnel and Billard 1991; Hymenomonadaceae, Fresnel 1994; Fresnel and Probert in press), where such swimmers may be considered as an ecological adaptation for dispersion of the species during the benthic phase.

Concerning sexual reproduction, Billard (1994) postulated that probably all coccolithophores (as most prymnesiophytes) had a heteromorphic life cycle with alternating haploid and diploid generations. Since this earlier synthesis, a wealth of new data has accumulated, confirming this hypothesis. Arguments for the existence of a haplo-diplontic life cycle in coccolithophores are based on the following type of evidence: (a) observation of "combination coccospheres" in field samples; (b) visualization of phase changes in culture; (c) electron microscopic examination of body scale types; (d) nuclear staining and relative chromosome counts; (e) flow cytometric analyses of relative ploidy levels; (f) observations of syngamy and meiosis. Conceptually, observation of syngamy and meiosis, such as described earlier for *Pleurochrysis* (Gayral and Fresnel 1983), provide the ultimate direct evidence of the existence of sexuality in coccolithophores but they are difficult to observe and furthermore restricted to species in culture. A recent study (Houdan et al. in press), using such material, illustrates these stages for the first time in *Coccolithus*, some 40 years after the historical paper by Parke and Adams (1960) reporting presence of two distinct phases in cultures of this coccolithophore. Using flow cytometric DNA analysis, they confirm that *Coccolithus* cells are diploid whereas the alternate holococcolithophorid stage is haploid; other species were analyzed in this study (see below) confirming the earlier hypothesis based on body scale morphologies, that heterococcolithophores are diploid whereas holococcolithophores are haploid. Furthermore, Houdan et al. (in press) report that the 18SrDNA sequence from a pure culture of *Crystallolithus braarudii* (the haploid phase of *Coccolithus* from temperate waters) is identical to a sequence of the same gene from the *Coccolithus* stage, providing genetic evidence that they belong to the same taxon (see Sáez et al. 2003 and Geisen et al. this volume, for new nomenclatural updates on this and other recently discovered pseudocryptic and cryptic species).

The fact that holococcolithophores are not autonomous species was suspected earlier by observations of combination cells (Kamptner 1941; Thomsen et al. 1991; Kleijne 1991). A growing number of illustrations of such cells, bearing both heterococcoliths and holococcoliths, and representing various species are now available (Cros et al. 2000b; Cortés and Bollmann 2002; Geisen et al. 2002; Saugestad and Heimdal 2002). Other combination cells featuring heterococcoliths and nannoliths (Alcober and Jordan 1997; Sprengel and Young 2000), or hetero-

coccoliths and aragonitic coccoliths (Cros et al. 2000a) have now been recently documented.

Heteromorphy is thus expressed in two different ways in each generation of a coccolithophore; (1) the morphology of the organism as a whole (e.g. motile vs. non-motile) and (2) the nature of the cell covering, and from evidence now available, the presence of heterococcoliths is in fact probably indicative of a diploid stage. Digenetic heteromorphic life cycles in coccolithophores show remarkable diversity with, so far, five different types, two of which were unsuspected in the earlier review by Billard (1994). Table 2 is a summary of the various types of life cycles known in heterococcolithophores.

The *Emiliana* life cycle

In *Emiliana*, non-motile heterococcolith-bearing cells (C-cells) alternate with motile scaly cells (S-cells); haplo-diploidy was confirmed by flow cytometric analyses (Green et al. 1996). As mentioned previously, the thin body scales of the S-cells differ from those of other prymnesiophytes. The cycle is the same in the closely related genus *Gephyrocapsa* (unpublished results) and is typical of the Noelaerhabdaceae. Nonmotile naked cells of *Emiliana* (N-cells) which are totally void of body scales are to be considered as mutant diploid stages having lost the ability to produce heterococcoliths (Paasche 2001).

A recent report (Laguna et al. 2001), describing peculiar S-cells, representing "a possible gametic stage" of *Emiliana*, must be considered with caution. These minute, bacteroid-like cells (see their Fig. 1) which grow on agar plates (where they can be maintained for over two years) are much smaller than the usual swimming S-cells of *Emiliana* (see Paasche 2001): although reported to be motile, the flagella were not illustrated, and the presence of scales was not confirmed.

Table 2. Summary of different life cycle types known in heterococcolithophores.

2 N (diploid) generation (HETEROCOCCOLITHS)	N (haploid) generation
Noelaerhabdaceae	non-calcifying motile stage
Coccolithaceae	HOLOCOCCOLITHS
Calcidiscaceae	
Helicosphaeraceae	
Papposphaeraceae	
Rhabdosphaeraceae	
Syracosphaeraceae	
Family incertae sedis (<i>Alisphaera</i> / <i>Canistrolithus</i>)	aragonitic coccoliths (<i>Polycrater</i>)
Ceratolithaceae	nannoliths (ceratoliths)
Pleurochrysidaceae	non-calcifying
Hymenomonadaceae	benthic stage

Heterococcolithophore-holococcolithophore life cycles

Well documented experimentally in members of the genera *Coccolithus*, *Calcidiscus* and *Coronosphaera* (Houdan et al. in press), heterococcolithophore-holococcolithophore life cycles may now be safely extended to all holococcolithophore "species" (previously classified in the Calyptrosphaeraceae) which are to be considered as haploid stages of certain heterococcolithophores. According to Sym and Kawachi (2000), the presence of a single plastid in holococcolithophores could also be an indication of haploidy. Details of the transformation from the diploid to the haploid phase probably vary between coccolithophores, but observation of combination cells point to its existence in other genera as well: *Pappomonas*, *Papposphaera*, *Wigwamma* (Thomsen et al. 1991; Østergaard 1993; Thomsen and Buck 1998), *Syracosphaera* (Cros et al. 2000b; Geisen et al. 2002; Saugestad and Heimdal 2002), *Acanthoica* and *Helicosphaera* (Cros et al. 2000b). Although they have not been reported since, combination coccospheres featuring *Algirosphaera robusta* and holococcoliths of *Sphaerocalyptra quadridentata* were illustrated by Kamptner (1941).

A recent study (Noël et al. 2002) reports an alternation between *Calyptrosphaera sphaeroidea* and a previously undescribed non-motile heterococcolithophore. In contrast to other studies, the life cycle was initiated from the holococcolithophore stage, the first organism isolated, and the change of phase to the coastal (?) heterococcolithophore stage was induced by alteration of environmental conditions in the culture; reversal to the motile *Calyptrosphaera*-stage was obtained using "opposite" environmental conditions. Both stages in this life cycle may be flagellate (e.g. species of *Syracosphaera*, *Coronosphaera* or *Helicosphaera*) which suggests that the advantages of such alternations are probably ecologically diverse within genera (or species) and remain to be established. Very few holococcolithophores are predominantly non-motile (Sym and Kawachi 2000) and such rare cases may indicate that their alternate heterococcolithophore stage could be restricted to coastal areas.

Families of typically oceanic coccolithophores concerned with this probably widely distributed life cycle are so far as follows: Helicosphaeraceae, Syracosphaeraceae, Rhabdosphaeraceae, Coccolithaceae, Calcidiscaceae and Papposphaeraceae (see Table 1). Considering that over 70 holococcolithophores have been observed or formally described in the literature, a large number of "missing couples" remain to be found, linking holococcolithophores with established species of heterococcolithophores. However, a number of the latter can be associated with two different holococcolithophore "species". Geisen et al. (2002) investigated this phenomenon and show that while some cases reflect ecophenotypic variations in the haploid phase (e.g. in *Helicosphaera carteri*), others are significant, and discrete variations in holococcoliths indicate either fine scale speciation (in *Coccolithus* and *Calcidiscus*) or cryptic speciation (e.g. in *Syracosphaera pulchra*) (see also Sáez et al. 2003 and Geisen et al. this volume).

Life cycles involving heterococcolithophores and stages with aragonitic coccoliths

Based on the observation of combination cells, this life cycle is so far unique to the genera *Alisphaera* and *Canistrolithus*. The alternate stage of these oceanic heterococcolithophores are various types of *Polycrater* (Cros et al. 2000a), a unique genus featuring small, aragonitic coccoliths (Manton and Oates 1980). *Polycrater galapagensis*, the only "species" formally described, is motile (Thomsen and Buck 1998) and so are certain species of *Alisphaera*.

Recent removal of *Alisphaera* and *Canistrolithus* from the Syracosphaeraceae (Kleijne et al. 2001) is consistent with the distinctiveness of their heterococcoliths (compared to *Syracosphaera*) and the existence of an alternate stage producing aragonitic structures. Cros et al. (2000a) suggest that the latter may replace holococcoliths in this particular life cycle.

Life cycle involving heterococcolithophores and stages with nannoliths

Combination cells of *Ceratolithus cristatus* featuring a single, large, horseshoe-shaped ceratolith (nannolith) inside a coccosphere of delicate, hoop-shaped heterococcoliths are well documented (see references in Cros et al. 2000b). Another association involving the hoop-shaped coccoliths with heterococcoliths (planoliths) of *Neosphaera coccolithophorpha* was first observed by Alcober and Jordan (1997), and Sprengel and Young (2000) provide the final direct evidence that all three calcareous structures are linked in the complex life cycle of *C. cristatus*. According to Young et al. (1998), the simplest hypothesis is that the ceratoliths are equivalent to holococcoliths and represent a haploid stage; the *Neosphaera*-type planoliths are normal heterococcoliths produced on diploid stages, the hoop-shaped coccoliths being alternative morphotypes produced by the same coccolith formation mechanism as planoliths. Living cells of *C. cristatus*, a warm water species of the upper photic zone, have not been observed since the earlier report of Norris (1965) and cultures of this organism are needed to test the above hypothesis based on the observation of combination coccospheres. *Ceratolithus cristatus* is the correct name for this unusual coccolithophore (see Young et al. 1998), which belongs to the monotypic family Ceratolithaceae.

Life cycles involving heterococcolithophores and non-calcifying stages

Historically, this is the best documented life cycle (see references in Billard 1994), and it is typical of coccolithophores inhabiting near-shore marine waters. While the diploid generation is a heterococcolithophore, with relatively small coccoliths (cricoliths or tremaliths), the haploid scaly generation is generally benthic, either pseudofilamentous (so-called *Apistonema*-stage of *Pleurochrysis*), forming pack-

ets (*Hymenomonas*) or palmelloid, i.e. embedded in mucilage (*Ochrosphaera*). Haploid cells of *Pleurochrysis* sp. have recently been observed as symbionts in a benthic foraminifer host, and were subsequently isolated in culture (Hawkins and Lee 2001). This suggests that other coastal coccolithophores could be temporary symbionts within heterotrophic hosts.

Whereas haploid stages of *Pleurochrysis* display a single type of unmineralized, rimless body scale (type 2, see above), both *Hymenomonas* (Fresnel 1994) and *Ochrosphaera* (Fresnel and Probert in press) produce two types of scales: the proximal layer is identical patternwise to type 2 scales of *Pleurochrysis*, while the distal layer is distinctive: these distal scales are rimmed, elevation and localization of the rim being species specific. These distal scales could be homologous to holococcoliths having lost their ability to calcify.

This life cycle, which has been substantiated by observation of syngamy and meiosis (*Pleurochrysis*) or by chromosome counts of stained nuclei (*Hymenomonas*, *Ochrosphaera*), is typical of the Pleurochrysidaceae and the Hymenomonadaceae (Table 1). The freshwater coccolithophore *Hymenomonas roseola* probably has the same type of life cycle as its marine counterparts (Fresnel 1994). *Jomonlithus*, a monotypic genus with distinctive heterococcoliths shows affinities with both the Pleurochrysidaceae and the Hymenomonadaceae with respects to its internal cellular organization (Inouye and Chihara 1983), but because of the absence of coccolithosomes it is tentatively placed in the Hymenomonadaceae (Table 1); its body scales are of the diploid type, but no alternate stage has yet been reported.

Anomalies exist in this life cycle, with one or the other generation potentially missing, and this is documented in both the above families (see Billard 1994). In any case, when a coccolithophore produces "naked" cells in culture, i.e. without apparent coccoliths, the cell covering should be checked with the TEM: if such cells produce type (1) body scales, then the cells have only lost the ability to calcify; if the cell covering consists of differently patterned scales, this may be indicative of a heteromorphic life cycle (Fresnel 1994).

To conclude, it is probably safe now to assume that the life cycle of all coccolithophores involves a haplo-diploid alternation of generations, each characterized by its cell covering, and each capable of asexual reproduction and of ensuring dispersal (Table 2). The advantages of the haplo-diploid life cycle, which are likely to broaden the ecological range of the species, are further discussed in Houdan et al. (in press). This contrasts with the situation in diatoms and dinoflagellates, other major groups in marine phytoplankton, where the life cycle is monogenetic (diploid and haploid, respectively). A distinctive feature in the biology of coccolithophores is that resting stages, i.e. hypnospores or cysts (whether sexual or vegetative) are seemingly absent. The pseudo-cysts described in the coastal genus *Ochrosphaera* could be an exception but they have not been observed in natural samples and may be opportunistic structures (as their mode of formation suggests), related to a favorable microenvironment in culture. It has also been suggested that the coccospheres of *Braarudosphaera bigelowii*, because they lack test perforations, are resting stages or cysts, but this remains to be confirmed.

In marine macroalgae, where digenetic life cycles are common, each generation with different ecophysiological traits may occupy a different ecological/seasonal niche. More experiments on coccolithophores in culture such as those initiated by Noël et al. (2002) are needed to determine life cycle strategies in this group and furthermore to determine which environmental factors trigger the change of phases in individual species. Another area of research would be to assess genetic expression in diploid and haploid phases in the life cycle of a coccolithophore, as has recently been done with both generations of the brown alga *Laminaria digitata* (Crépineau et al. 2000). The possibility that such changes are partly controlled by endogenous regulation (biological clock) cannot, however, be ruled out (see discussion in Houdan et al. in press).

Taxonomic concepts

Progress in the phylogeny of coccolithophores

Phylogenetic reconstructions of the Haptophyta inferred from plastid (*rbcL*) (Fujiwara et al. 2001) or nuclear encoded gene sequences (18S ribosomal DNA) (Edwardsen et al. 2000; Sáez et al. this volume), along with supporting morphological data are beginning to provide an objective framework within which to comment on the systematics of extant coccolithophores.

In the monophyletic Prymnesiophyceae, coccolithophores constitute a clade including non-calcifying genera such as *Isochrysis*. Re-instatement of the Isochrysidales (Edwardsen et al. 2000) is supported by significant ultrastructural and biochemical data. Further support is provided by body scale characteristics and by the specific life cycle demonstrated in the calcifying Noelaerhabdaceae. Species in the non-mineralized Isochrysidaceae are now considered as coccolithophores having secondarily lost the ability to produce coccoliths (Fujiwara et al. 2001).

Within the remaining coccolithophores, a number of clades are recognized which support certain current families (see Sáez et al. this volume.): Cocco-lithaceae, as a sister group to Hymenomonadaceae and Pleurochrysidaceae; Helicosphaeraceae. The Syracosphaeraceae, represented by *Coronosphaera mediterranea* (monothecate) and *Syracosphaera pulchra* (dithecate) may not be a natural group but more representatives need to be sequenced. *Algirosphaera robusta* (Rhabdosphaeraceae) is distinguished by genetic analyses and, as mentioned earlier, its apparently monomorphic body scales are quite distinctive. Nevertheless, the fine structure of *A. robusta* and its coccolith structure show affinities to *S. pulchra* (Probert et al. in press).

The case of *Reticulosphaera*, a genus (two species described) of photophagotroph meroplasmodial protists must be mentioned here. Although originally placed in the Heterokontophyta (Grell et al. 1990), it is considered by some authors as an aberrant haptophyte (Cavalier-Smith et al. 1996), following analysis of the 18S rRNA gene sequence of *R. japonensis*. The latter appears as a sister taxon to *Pleurochrysis* in the tree of Edwardsen et al. (2000). *Reticulosphaera* spe-

cies differ radically from haptophytes in external body form, and the ultrastructural features of the type, *R. socialis*, the only species examined (Grell et al. 1990), are not prymnesiophycean. Pending further studies, it seems unreasonable to include the amoeboid Reticulosphaeraceae in the Coccolithales.

The major taxonomic groups featuring extant heterococcolithophores (Table 1) have so far been based mostly on coccolith architecture. Some currently recognized orders (i.e. Isochrysidales) or families (see above) are supported by genetic data, whereas uncertainties remain for others. A more natural classification system, namely at the ordinal level, must await further analyses of nucleotide sequences of appropriate genes.

Nomenclatural problems

Problems arising from the existence of heteromorphic life cycles in coccolithophores should be addressed. When the alternate phase is non-calcifying, naming these somewhat inconspicuous haploid generations has rarely been an issue except for *Pleurochrysis* where the distinctive pseudofilamentous stages of certain species was sometimes designated as the *Apistonema*-stage; conversely, the genus *Cricosphaera* (a junior synonym of *Pleurochrysis*) was in use for species of *Pleurochrysis* where the benthic stage had not been observed (see Fresnel and Billard 1991). Such practice in the Pleurochrysidaceae has now been generally abandoned.

The situation is different when the alternate (haploid) phase is a distinctive coccolithophore, with coccoliths visible under the light microscope. In the past, both stages were named according to the Linnean system since they were considered as autonomous species. Such is the case with most holococcolithophores (see Jordan and Green 1994) which are presented as distinct taxa in phytoplankton manuals (Heimdal 1993). So far, in all situations where combinations have been reported, the name of the heterococcolithophore (diploid phase) has had priority over the alternate (haploid phase). In agreement with Cros et al. (2000b) we urge that, as far as possible, nomenclature should be based on the heterococcolith phase for the generic name, while the first described epithet should be applied (whether it belongs or not to the heterococcolith phase). As suggested by Young et al. (1998), the original name of the alternate phase could continue to be used in an informal non-Linnean sense. It must be noted that in marine macroalgae with heteromorphic life cycles, there is no standing rule. In the brown algae, for instance, the name of the species is either applied to the sporophytic (diploid) generation (e.g. *Laminaria digitata*) or to the gametophytic (haploid) generation (e.g. *Cutleria multifida*). Similar cases may be found in the red or the green algae. The "trend" in macroalgae is that the binomial with priority corresponds to the dominant, more conspicuous generation, which was formally described first, according to the rules of the ICBN. Nevertheless, for clarity's sake in the complex taxonomy of coccolithophores, it would be wise to apply the informal rule mentioned above.

When an undescribed holococcolithophore is discovered in combination with a previously known heterococcolithophore, a recent practice has been simply to

designate the alternate stage as (HO), e.g. *Acanthoica quattrosipina* (HO) for the holococcolithophore stage of this species (see Cros et al. 2000b). Furthermore, recent diagnoses of new species do not account for or mention the alternate stage and its coccoliths, even when the latter is recorded, e.g. in *Syracosphaera delicata*, a species recently described (Cros et al. 2000b). It would be nevertheless preferable in such diagnoses to furnish some information on the alternate stage of the heterococcolithophore (when available) to allow unambiguous communication, in nanofloral analyses, for instance. For similar purposes, it seems necessary, as a first step, to formally name novel (apparently independent) holococcolithophores (as done by Sym and Kawachi 2000), the ultimate goal being to eventually link them to existing (or new) heterococcolithophore species, via culture studies or observation of combination cells.

The possibility also exists that some life cycles in coccolithophores might be isomorphic, i.e. that both generations could be morphologically identical. Isomorphic life cycles (considered more primitive) are known in different macroalgal groups with alternations of generations, so this possibility cannot be ruled out in coccolithophores (or Haptophyta as a whole). In short, formal knowledge of both phases of a coccolithophore species is important since each phase may have different strategies and these have great implications for understanding the ecology of the group.

Conclusions

In recent years knowledge of the biology of coccolithophores has progressed thanks to culture studies and meticulous observations of coccospheres in wild samples. Concerning the cytology of the cells, uncertainties remain, relative, for instance, to the heterotrophic, aplastidic Papposphaeraceae which need to be cultured and examined with the TEM, as well as groups producing nannoliths (e.g. Braarudosphaeraceae) whose status need to be confirmed. Studies of cell coverings in coccolithophores are highly informative, each generation of the life cycle being characterized by a distinctive type of scale or coccolith/nannolith. The mode of synthesis of holococcoliths still remains enigmatic, while diversity is becoming apparent in details of heterococcolith production. Studies of the flagellar apparatus have informative value but few coccolithophores have been thoroughly examined in this sense. Phylogenetic analyses are in progress which, in accordance with morphological or biochemical data, should allow in the future a more natural classification of the coccolithophores. Their haplo-diploid, typically heteromorphic life cycle, shows remarkable diversity and it can now safely be extended to a number of representative families, spanning the diversity of the group. Ecophysiological studies devoted to each generation of a single species and determination of the environmental factors triggering phase changes, should be a promising area of research in order to better understand the distribution and ecology of coastal or oceanic coccolithophores.

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Calcification in coccolithophores: A cellular perspective

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Summary

Knowledge of the mechanisms of calcification in different species of coccolithophores and the interactions between calcification and other cellular processes is required for further understanding the regulation of a key component of inorganic carbon flux in the oceans. In particular the functions of calcification in relation to photosynthesis and nutrient acquisition are still debated. Increased understanding of the cellular regulation of calcification is also required to accurately predict the responses to elevated atmospheric CO₂ on a global scale. Moreover, transport processes in delivery of substrates for calcification will improve our ability to interpret isotopic fractionation in the fossil record that is increasingly being used as a proxy for past climatic conditions. Advances in single cell physiology and molecular biology are already contributing significantly to the study of calcification at the cellular level. The application of genomics approaches should, in the longer term, contribute further to the goal of understanding of how cellular functions contribute to processes at ecosystem and global levels.

Introduction

The intricate shapes of coccoliths and the frequently observed high production rates of calcite by the unicellular coccolithophorid algae have attracted the interests of ecologists, physiologists and cell biologists for many years. The coccolithophorid phytoplankton are responsible for the largest production of calcite on earth. The global occurrence of coccolithophores and the ability of certain species to form extensive blooms in oceanic or coastal surface waters under certain conditions suggest that they need to be considered in models of ocean productivity. The formation of CaCO₃ by the calcifying life cycle stages of coccolithophores and its removal from surface waters by sinking has important implications for the flux of inorganic carbon in the oceans (Westbroek et al. 1993, 1994; see Rost and Riebesell this volume).

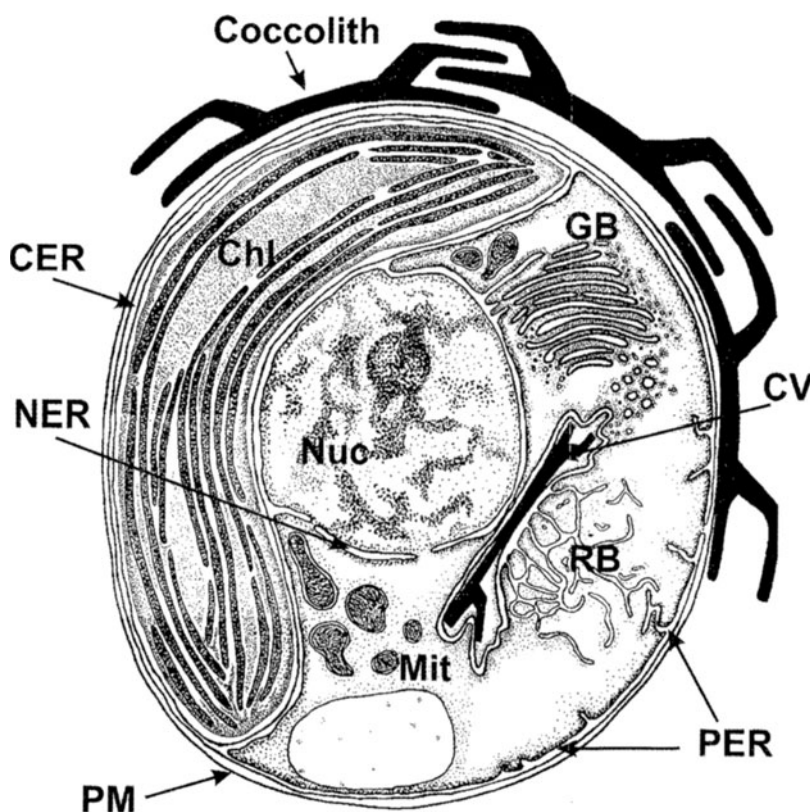


Fig. 1. Diagrammatic representation of the endo-membrane system in a coccolithophore cell, drawn with reference to Manton and Leedale (1969) and Klaveness (1972). Nuc, nucleus; Mit, mitochondria; Chl, chloroplast; PM, plasma membrane; GB, Golgi body; CV, coccolith vesicle; RB, reticular body; PER, peripheral endoplasmic reticulum; NER, nuclear endoplasmic reticulum; CER, chloroplast endoplasmic reticulum.

To understand fully the mechanism of coccolithophorid calcification at a cellular level in relation to function it is necessary to elucidate the different steps and pathways involved in the formation of coccoliths. Calcification ultimately involves the precipitation of CaCO_3 from Ca^{2+} and CO_3^{2-} ions in solution. However, despite much intense investigation, the precise mechanisms and transport routes of substrates leading up to the precipitation reaction are still unclear. Here we outline aspects of the current state of knowledge concerning the cellular physiological mechanisms and functions of calcification, paying particular attention to current

uncertainties and controversies and indicating where advances are likely to be made. The reader is referred to recent in-depth reviews by Paasche (2001) on the physiology and general biology of coccolithophore calcification and by Young et al. (1999) on coccolith structure and diversity and crystallization mechanisms.

Calcification and cellular structures

Cell ultrastructure has been well characterized with respect to the mechanism of calcification in a few species of coccolithophores, notably *Emiliania huxleyi*, *Coccolithus pelagicus* and *Pleurochrysis carterae* (Van der Wal et al. 1985; Manton and Leedale 1969; Marsh 1994). Fig. 1 summarizes the main intracellular components involved in calcification in the most intensively studies species, *E. huxleyi*.

E. huxleyi is typical of species that produce complex heterococcolith crystals in intracellular compartments (Young et al. 1999) and which dominate the modern coccolith flora in terms of global occurrence. Intracellular coccolith production requires the maintenance of significant sustained net fluxes of Ca^{2+} and inorganic carbon (Ci) from the external medium to the intracellular membrane-bound, Golgi-derived calcifying compartment (the coccolith vesicle, CV). Certain species such as *Coccolithus pelagicus* produce heterococcoliths or more simple holococcolith calcite crystals in different phases of their life cycle (e.g. Cros et al. 2000). While published evidence reports the production of holococcoliths on the external surface of the cell (Rowson et al. 1986; Young et al. 1999), it is not clear whether they may also be produced in internal compartments. The following discussions, however, refer to the internal production of heterococcoliths.

Challenges in coccolithophore cell biology

The physical separation of the calcification compartment within the cell allows the creation of microenvironments in which calcite precipitation can be controlled. While several species of algae produce microenvironments on their cell surface that favor calcite formation (Borowitzka 1982; McConnaughey and Whelan 1996), internal calcite precipitation requires the uptake of Ci and Ca^{2+} and their transport to the coccolith vesicle (Berry et al. 2002; Brownlee et al. 1994; Paasche 2001).

While much of our knowledge of the cell physiology and cell biology of coccolithophores has been gained from studies of *E. huxleyi*, considerable diversity is likely in the cellular mechanisms of calcification amongst species, strains and life cycle stages (see Billard and Inouye this volume). It is becoming increasingly important to identify the extent to which common functions and mechanisms of calcification can be defined. An example of the divergence in calcification mechanisms between species at the cellular level can be seen from the different involvement of intracellular membranes and polysaccharides in calcification in *E.*

huxleyi and *P. carteri*. In *E. huxleyi*, the coccolith vesicle is closely associated with a membranous structure known as the reticular body which is thought to be essential for supply of calcification precursors to the CV (De Jong et al. 1976). In contrast, the reticular body appears to be absent in *P. carteri* (Marsh 1994). It has been proposed (Paasche 2001; Marsh and Dickinson 1997) that this may relate to the intricate involvement of a particular type of high capacity Ca^{2+} -binding polysaccharide (PS2), that is essential for calcification in *P. carteri* and is probably involved in the delivery of Ca^{2+} from the Golgi to the CV. PS-2 is not found in *E. huxleyi* (Corstjens et al. 1998) which produces a different coccolith-associated polysaccharide (coccolith polysaccharide, CP) that is involved in regulating coccolith crystal growth (De Jong et al. 1976).

Other major challenges in coccolithophore biology lie in relating relevant features of the cell biology of calcification, photosynthesis and nutrient acquisition to ecophysiology, distribution and succession of coccolithophores in the field and their impact on air-sea CO_2 exchange (see Rost and Riebesell this volume; Buitenhuis et al. 2001; Crawford and Purdie 1997). Moreover, an understanding of the mechanisms of calcification is fundamental to interpreting isotope discrimination data from the fossil record in relation to paleoclimate and productivity. Finally, the cellular mechanisms by which elevated CO_2 can affect cell growth and calcification rate need to be elucidated in order to understand the impact of global increases in CO_2 on inorganic carbon fluxes (Zondervan et al. 2001, 2002; see Rost and Riebesell this volume).

Functions of calcification

Advances in understanding the mechanism of calcification are likely to shed light on its function. A variety of protective, metabolic and adaptive functions for calcification have been proposed. Phytoplankton are particularly vulnerable to grazing and pathogen attack and many species have evolved protective cell coverings based on silica (diatoms) or organic carbon (e.g. dinoflagellates). Indirect evidence for a protective role for coccoliths comes from the observation that calcifying cells of *E. huxleyi* adjust their calcification/cell division rates in order to maintain at least one complete layer of coccoliths (15 per cell) around the cell surface, even under growth limiting conditions, such as low light (Paasche 1999). However, a function in addition to protection, at least in *E. huxleyi* (Paasche 2001), is suggested by the observation that under high light conditions that are not limiting to growth, calcifying cells produce more coccoliths than are needed for a single layer covering and may even shed excess coccoliths into the medium (Paasche 1999). An efficient protective role is not supported by studies of grazing by zooplankton that have shown comparable high rates of grazing of calcified and non-calcified cells (Harris 1994). Moreover, direct measurements of copepod gut pH showed no differences between individuals feeding on diatoms, dinoflagellates or coccolithophores, suggesting that calcite did not significantly affect the digestive process (Pond et al. 1995). A ship-board study by Nejstgaard et al. (1997)

suggested that reduced micro-zooplankton population size in response to meso-zooplankton grazing could give rise to blooms of *E. huxleyi*, implying significant control of population size by microzooplankton grazers.

The demonstration of high rates of viral infection of calcifying *E. huxleyi* cells (Bratbak et al. 1996; Wilson et al. 2002) suggests that coccoliths do not afford significant protection against viral attack. The role of coccolith production in protection against bacterial attack has not been investigated. Calcite coccolith plates may provide a protective surface covering with a low organic carbon content analogous to the silica-rich cell walls of diatoms. Such an adaptation would potentially provide advantages over phytoplankton that form organic carbon-rich cell walls (e.g. dinoflagellates), particularly under conditions where light or carbon was limiting for photosynthesis and growth. However, the correlation between coccolithophore blooms and high light intensities (Nanninga and Tyrrell 1996) is not consistent with this function.

Metabolic functions for calcification have been suggested by various studies. The production of coccoliths has been shown to increase sinking rates, which may facilitate movement of cells to deeper waters with potentially higher nutrient levels (Lecourt et al. 1996). *E. huxleyi* has also been shown to have a uniquely high affinity inducible uptake system for phosphate (Riegman et al. 2000). This correlates well with the occurrence of blooms in phosphate-poor waters (Tyrrell and Taylor 1996). Moreover, both calcification and membrane-bound cell surface alkaline phosphatase activity showed stimulation by low nutrient availability in a low calcifying strain of *E. huxleyi* (Riegman et al. 2000) raising the question of whether calcification is related directly to acquisition of phosphate.

A further outstanding feature of *E. huxleyi* is the absence of inhibition of photosynthesis at high irradiances (higher than $750 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) that would lead to photoinhibition in other phytoplankton species (e.g. Nimer and Merrett 1993; Nielsen 1997; Nanninga and Tyrrell 1996). Resistance to photoinhibition is also found in other coccolithophore species such as *Pleurochrysis* spp. (Israel and Gonzalez 1996) but appears to be specific to coccolithophores since other members of the haptophyte algae such as *Isochrysis galbana* are significantly photoinhibited at high light levels (Grima et al. 1996). *E. huxleyi* blooms are frequently observed in shallow mixed waters and surface oceanic waters in high light conditions where photoinhibition may limit the growth of other phytoplankton species (Nanninga and Tyrrell 1996). This suggests that calcification may serve a protective role under high light conditions, either by direct shielding of the underlying cell from light or by providing a metabolic sink for excess reductant produced by photosynthetic electron transfer. However, while these hypotheses have still not been tested rigorously, the limited available data does not support a photo-protective role. Nanninga and Tyrrell (1996) also showed that decalcified cells of a calcifying strain of *E. huxleyi* remained resistant to photoinhibition indicating that coccoliths do not provide shading against excess light levels. In the same study, a non-calcifying strain of *E. huxleyi* was also shown to be resistant to photoinhibition, suggesting that calcification does not provide a metabolic protection against high light levels. More experiments monitoring photosynthesis in calcifying and non-calcifying cells at high light levels under varying conditions of carbon

availability may shed further light on whether calcification serves any possible photo-protective role under more extreme conditions.

Calcification and carbon acquisition

Photosynthesis by marine phytoplankton requires acquisition of C_i from seawater. Ribulose biphosphate carboxylase oxygenase (Rubisco) fixes C_i into organic carbon using CO_2 as its substrate. Rubisco possesses both carboxylase and oxygenase activities. Oxygenase activity can be significant at high O_2 levels, leading to photorespiration (Falkowski and Raven 1997). The Rubisco specificity factor τ is a measure of the relative carboxylase/oxygenase activities. Both τ and the affinity constant of Rubisco for CO_2 (K_m) vary considerably between species (Raven 1997; Tortell 2000) and K_m may be significantly higher than the ambient seawater CO_2 concentration. Values of phytoplankton K_m and τ have been related to decreases in atmospheric pCO_2 over geological time with a trend towards higher affinity for CO_2 and carboxylase activity with decreasing pCO_2 levels since the appearance of the green algae approximately 500 Myr ago (Tortell 2000). The coccolithophores appeared in the geological record approximately 200–250 Myr ago when seawater CO_2 levels were relatively low and have τ values intermediate between the more ancient green algae and the more recent diatoms (Tortell 2000).

Species that rely on the diffusion of CO_2 from seawater for photosynthesis may potentially be limited by seawater CO_2 availability (Riebesell et al. 1993) and a number of unicellular algae appear to be able to elevate CO_2 at the site of Rubisco action in the chloroplast. Indeed a negative correlation between Rubisco τ and carbon concentrating factor (i.e. the ratio of measured intracellular C_i relative to extracellular C_i concentration) has been reported (Tortell 2000). Carbon concentrating mechanisms (CCMs) based on direct HCO_3^- and/or CO_2 active transport at the chloroplast or plasma membrane have been described for several marine phytoplankton species (Colman et al. 2002; Huertas et al. 2002; Matsuda et al. 1998, 2002; Morel et al. 2002). These may also involve the action of external carbonic anhydrase to facilitate the production of CO_2 at the cell surface and/or chloroplast carbonic anhydrase, which may facilitate CO_2 production from HCO_3^- in the chloroplast (Raven 1997).

In calcifying coccolithophore cells the net rates of fixation of C_i into calcite and CO_2 into organic carbon can be comparable (i.e. calcification/photosynthesis (C/P) ratios close to unity, ignoring losses due to dissolution of calcite and respiration of organic carbon). Calcification thus clearly represents a major flux of C_i through these cells. It may be reasonable to assume that this C_i flux may have a significant impact on cellular carbon metabolism and photosynthesis. However, while substantial data exists showing interactions between calcification and photosynthesis (Paasche 2001), a direct role of calcification in the acquisition of C_i is being questioned by some recent studies.

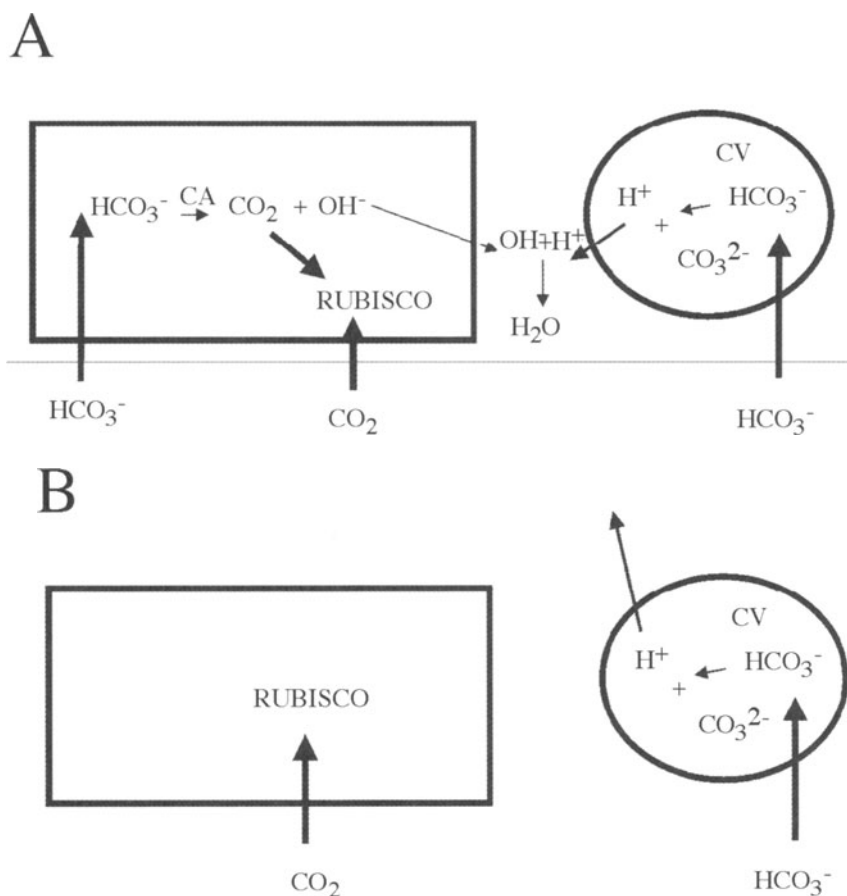


Fig. 2. Simplified schemes showing alternative hypotheses for the fixation of Ci into calcite and photosynthesis. A. HCO_3^- use by calcification results in the production of H^+ while photosynthesis can utilize either CO_2 or HCO_3^- . HCO_3^- use by photosynthesis results in net alkalization, which may be offset by H^+ produced during calcification. B. H^+ produced by calcification are not utilized by photosynthesis or other H^+ -requiring processes. Photosynthesis uses CO_2 primarily as the Ci source. In both cases photosynthesis provides the main energy source for processes involved in calcification (see text for details).

There is good evidence that HCO_3^- is the external Ci source for calcification in coccolithophores (see Paasche 2001; Berry et al. 2002 for recent reviews). H^+ produced during the formation of carbonate in the coccolith vesicle (Fig. 2) may potentially be used to enable production of CO_2 from Ci accumulated as HCO_3^- in the chloroplast (Fig. 2A). Calcification may therefore have provided coccolithophores with an evolutionary advantage under conditions where CO_2 availability may be limiting for photosynthesis. However, it is interesting to note that while

coccolithophores probably arose during a period of relatively low CO_2 availability, they have persisted through the geological past when seawater CO_2 was significantly higher than present (Tortell 2000; Paasche 2001). This suggests that factors unrelated to CO_2 acquisition may have favored the retention of coccolith production.

The source of Ci for photosynthesis in coccolithophores is less clear. There is evidence that *E. huxleyi* is able to accumulate Ci to levels approximately ten-fold higher than those in seawater (Sekino and Shirawa 1994) though other reports have indicated no accumulation in *P. carterae* (Israel and Gonzalez 1996) or *E. huxleyi*, (Nimer and Merrett 1992). Interestingly, the approximately 10-fold accumulation of Ci shown by Sekino and Shirawa (1994) appeared not to be utilized by photosynthesis. Photosynthesis appeared to use external CO_2 while calcification was shown to use HCO_3^- (Fig. 2B).

Experiments specifically designed to test the role of calcification in photosynthetic carbon acquisition have yielded mixed results and there is evidence both for and against a function of calcification in the direct facilitation of photosynthesis. The frequently observed comparable molar rates of organic carbon fixation and calcite production are consistent with a close functional relationship. Moreover, the overproduction and shedding of excess coccoliths by *E. huxleyi* (Paasche 1999) suggests a role or roles in addition to a protective one. While coccolithophores possess chloroplast carbonic anhydrase (Nimer et al. 1994; Quiroga and Gonzalez 1993) they do not appear to possess external carbonic anhydrase (Nimer et al. 1994, 1999), suggesting that they lack a carbon concentrating mechanism based on external conversion of HCO_3^- to CO_2 and active CO_2 uptake. However, Elzenga et al. (2000) have shown that presence or absence of external CA may be strain-specific. They provided evidence, based on ^{14}C isotopic disequilibrium experiments, for either external CA-catalyzed conversion of HCO_3^- to CO_2 in some strains of *E. huxleyi* or exclusive use of free CO_2 in other strains.

Experiments using ^{14}C as a tracer for calcification have shown that supply of Ci to photosynthesis required HCO_3^- utilization by calcification in both *P. carteri* (Israel and Gonzalez 1996) and *E. huxleyi* (Buitenhuis et al. 1999). Photosynthesis in *E. huxleyi* at low external Ci (HCO_3^-) (<0.5 mM) was shown to be dependent on CO_2 diffusion but became saturated at relatively low CO_2 under these conditions. However, increasing external HCO_3^- above 0.5 mM was shown to increase photosynthesis dramatically at saturating external CO_2 . In parallel, calcification was completely inhibited by HCO_3^- < 0.5 mM. While these results could be interpreted in terms of the operation of a relatively low affinity HCO_3^- transport system directly supplying HCO_3^- for photosynthesis and calcification, they also suggest that calcification could stimulate photosynthesis. Explaining these results simply in terms of CO_2 supply to photosynthesis is difficult since there appears to be an additional requirement for HCO_3^- even at CO_2 concentrations where photosynthesis is saturated. It is possible that calcification is able to supply a factor or factors that can alleviate this saturation.

The direct contribution of calcification to photosynthesis in *E. huxleyi* has, however, been called into question by experiments that have directly inhibited calcification by removal of Ca^{2+} from the growth medium (Herfort et al. 2002). In

these experiments photosynthesis was shown to be unaffected by removal of Ca^{2+} whereas calcification could be inhibited completely. This is in agreement with earlier studies by Paasche (1964) but contrasts with those of Nimer et al. (1996) who showed that at relatively high external pH, Ca^{2+} removal had similar effects on photosynthesis and calcification. The difference between the results of Nimer et al. and Paasche could at least be partially explained by the higher external medium pH values used by Nimer et al. where CO_2 availability would be low. Herfort et al. (2002) compared photosynthesis and calcification in calcifying cultures with those that had been adapted to Ca^{2+} -free conditions for several generations, whereas Nimer et al. (1996) measured short-term ^{14}C fixation immediately after Ca^{2+} removal. It is possible that the adapted cultures of Herfort et al. (2002) may have changed their carbon acquisition strategy in response to long-term Ca^{2+} removal. If this is so it indicates the presence of significant plasticity of coccolithophores with respect to carbon acquisition mechanisms. Surprisingly, the calcifying cultures of Herfort et al. (2002) showed ^{14}C C/P ratios approaching values of 2.0, significantly higher than C/P ratios reported in a variety of other studies (e.g. Paasche 1964; Nimer et al. 1996). Interestingly, a similar lack of dependence of photosynthesis on calcification has been reported recently in the coral species *Stylophora pistillata* (Gattuso et al. 2000). The experiments of Herfort et al. (2002) also indicated that Ci uptake was biphasic and sensitive to the anion transport inhibitors DIDS and SITS. At low (< 0.5 mM) Ci , the operation of a high affinity transporter was suggested by measurements of ^{14}C uptake and O_2 evolution. However, in sharp contrast with the results of Buitenhuis et al. (1999; see above), Herfort et al. (2002) showed that photosynthesis was higher at 0.25 and 0.5 mM Ci than at 1.0 mM Ci in the external medium. Clearly more work is required to resolve these issues.

Measurements of stable carbon isotope discrimination also indicate that *E. huxleyi* can accumulate Ci but that calcification is not used directly to supply photosynthesis with carbon (Rost et al. 2002). In this detailed study the relationship between specific growth rate μ , CO_2 and $^{13}\text{C}/^{12}\text{C}$ isotope fractionation of particulate organic carbon (POC) fixation ϵ_p deviated significantly from the theoretical inverse relationship between ϵ_p and μ/CO_2 that would be expected for diffusive CO_2 uptake, making the scheme presented in Fig. 2B unlikely. Moreover, ϵ_p was more influenced by photon flux density (PFD) and light regime, than by growth rate, showing higher discrimination under higher PFD and photoperiod, indicating greater use of CO_2 under these conditions. These results also showed that increased C/P ratios gave rise to higher isotopic discrimination in organic carbon. Since HCO_3^- is more enriched in ^{13}C than CO_2 this indicated that direct HCO_3^- involvement in photosynthesis *via* calcification was not occurring. The results were consistent, however with HCO_3^- use for calcification, showing less ^{13}C enrichment in calcite compared with POC.

It has been proposed that if calcification serves to reduce the energetic cost of carbon acquisition then the cost of calcification should be itself minimal (Anning et al. 1996; Berry et al. 2002). Analysis of transport costs has indicated that calcification could require only a small percentage of total energy costs of carbon ac-

quisition if Ca^{2+} can enter the coccolith vesicle by a route that does not involve significant diffusion of free Ca^{2+} through the cytoplasm (Anning et al. 1996; Berry et al. 2002). However, the costs of calcification are not confined to those involved in the trans-membrane transport of reactants and products. Significant biochemical costs may be incurred, for example in the Golgi-localized production of coccolith-associated polysaccharides that appear to be involved in regulation of calcification in the coccolith vesicle (Marsh 1994, 1996; Van Emburg et al. 1986). Nanninga et al. (1996), using an immunochemical assay for soluble secreted polysaccharides of *E. huxleyi* showed that these probably represented only a small fraction of total primary production. However, coccolith-associated polysaccharides are produced in far greater abundance. A detailed biochemical analysis of the structure, turnover and Ca^{2+} -binding properties of the major coccolith-associated polysaccharides in *P. carterae* allows an estimate to be made of the costs associated with their production. Marsh (1994, 1996) showed that the major Ca^{2+} -binding polyanion polysaccharides of *P. carteri* were not recycled. Instead, all of the ^{14}C -labelled polyanions in pulse-chase experiments were secreted during calcification. Isolated coccoliths contain approximately 6 moles of Ca^{2+} per mole of polyanion carboxyl group (Marsh 1994, 1996). In *P. carterae* the major calcification-associated polysaccharide (PS-2) has a carbon/COOH ratio of approximately 3.0 (Marsh 1994, 1996). If PS-2 is involved in Ca^{2+} delivery to the site of calcification without any turnover and if all precipitated Ca^{2+} were transported to the coccolith in polyanion complexes, then 3 moles of carbon would be fixed into polysaccharide and secreted for every 6 moles of Ca^{2+} fixed into calcite. For a coccolith cell with a C/P ratio of 1.0 this means that up to 50% of total organic carbon fixation may be required for production of coccolith-associated polysaccharides. This represents a significant energetic cost, larger in fact than the combined energy calculated for Ci and Ca^{2+} transport (Brownlee et al. 1994; Anning et al. 1996). Clearly this does not argue strongly for a cost-saving energetic role for calcification.

Cellular Ca^{2+} transport and Sr/Ca ratios

Sr/Ca ratios of calcite in the fossil record have been proposed as useful climate proxies (e.g. Stoll et al. 2002; this volume) and have been shown to correlate with temperature and growth rate in cultures (Stoll et al. 2002). In abiogenic calcification, higher calcification rates produce higher calcite Sr/Ca ratios (Stoll et al. 2002, this volume). Kinetic effects on Sr/Ca ratios would dominate in an open system where the rate of supply of ions exceeded the rate of calcite precipitated (Stoll et al. this volume). Measurements from coccoliths suggest that high observed Sr/Ca ratios could not be explained by the kinetics expected from an open precipitation system. Instead they could only be explained either by surface enrichment of Sr^{2+} at the site of calcification or precipitation from a fluid with a Sr/Ca ratio approximately twice that of seawater (Stoll et al. this volume). Cellular control of Sr/Ca ratios would dominate if the cell behaved as a closed system with respect to calcification i.e. if most or all of Sr^{2+} and Ca^{2+} taken up by the cell crossed the plasma membrane and was used in calcification without large recy-

clinging fluxes across the plasma membrane. Rickaby et al. (2002) have proposed that reduced selectivity of transporters for Sr^{2+} relative to Ca^{2+} may explain the observed Sr^{2+} enrichment in coccolith calcite. A complete understanding of the transport pathway for Ca^{2+} to the coccolith vesicle will be critical for further analysis of this problem.

Berry et al. (2002) discussed the possible role of endocytotic, vesicle-mediated influx of Ca^{2+} to the site of calcification. Significant recycling of plasma membrane occurs in calcifying cells, providing a potential pathway for Ca^{2+} entry. However, direct attempts in our laboratory to demonstrate the existence of a fluid-phase endocytotic uptake system in coccolithophores have so far been unsuccessful. Ca^{2+} entry into calcifying coccolithophores is thus more likely to occur via Ca^{2+} -permeable channels in the plasma membrane.

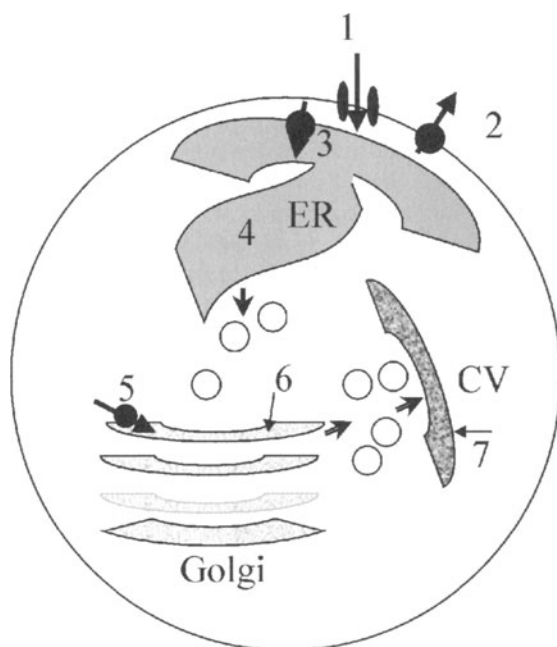


Fig. 3. Potential selectivity filters for Ca^{2+} transport to the coccolith vesicle. 1. Entry into the cytosol through plasma membrane Ca^{2+} -permeable channels. 2. Extrusion via Ca^{2+} -ATPase efflux pumps. 3. Entry into the endoplasmic reticulum (ER) through ER Ca^{2+} -ATPase. 4. Selective binding to Ca^{2+} -binding proteins in the ER. 5. Ca^{2+} transport into the Golgi driven by the H^+ electrochemical gradient across the Golgi membrane. 6. Selective binding to Ca^{2+} -binding polysaccharides in the Golgi. 7. Kinetic effects during precipitation of CaCO_3 in the coccolith vesicle (CV).

Consideration of the possible selectivity filters represented by putative membrane channels and pumps likely to be involved in Ca^{2+} delivery to the coccolith vesicle and cellular Ca^{2+} homeostasis suggests that relative transport efficiencies of Sr^{2+} and Ca^{2+} may vary considerably with the type of transporter under consideration. In most eukaryotic cells Ca^{2+} entry occurs down its electrochemical potential gradient through Ca^{2+} -permeable or Ca^{2+} -selective channels (Fig. 3).

Significant variability in relative permeability to Sr^{2+} and Ca^{2+} exists for animal Ca^{2+} channels. For example, currents through Ca^{2+} -permeable channels in jellyfish striated muscle cells show higher permeation of Ca^{2+} compared with Ba^{2+} or Sr^{2+} (Lin and Spencer 2001). In contrast, the relative permeability to Ca^{2+} , Ba^{2+} or Sr^{2+} of cloned alpha subunits of Ca^{2+} channels varies with the subunit type, showing higher permeability to Ba^{2+} or Sr^{2+} than to Ca^{2+} (Bourinet et al. 1996). The permeation properties of plant Ca^{2+} channels have been considerably less well studied. However, the few plant Ca^{2+} -permeable channels that have been studied to date have shown higher permeability to Ba^{2+} than to Ca^{2+} (e.g. Very and Davies 2000). Given the phylogenetic distance between haptophyte algae and higher plants, a plant-based comparison of Ca^{2+} channel properties is unlikely to be very revealing. The above examples suggest that channel-mediated entry of Ca^{2+} into the coccolithophore cell may have a significant influence on the Sr/Ca ratio of the calcifying fluid. So far, we know little about the plasma membrane properties of coccolithophores, let alone the types of Ca^{2+} channel present.

Further analysis of the likely transport pathways and mechanisms of Ca^{2+} homeostasis in coccolithophores should indicate the nature of any further selectivity filters for Sr^{2+} and Ca^{2+} transport and should allow the consideration of whether coccolithophore calcification occurs in a predominantly closed or open system. Rickaby et al. (2002) have proposed that a rate-dependent discrimination against the larger ion (Sr^{2+}) may explain the higher Sr/Ca ratios at higher growth rates. This could occur both via a pump or channel-mediated transport. Thus, stronger non-covalent binding of Ca^{2+} by a transport molecule and more efficient transport at lower ion concentrations was proposed. At higher rates of transport or higher concentrations of substrate molecules, Sr^{2+} transport would increase proportionately more than Ca^{2+} transport leading to increased Sr/Ca ratios. In a similar manner, binding site selectivity for Ca^{2+} against Sr^{2+} of a putative Ca channel may become lower at higher transport rates. The simplest model for Ca^{2+} transport during calcification involves channel-mediated influx of Ca^{2+} into the cytosol across the plasma membrane followed by accumulation into the Golgi. While there are reasons to suppose that this simple pathway is unlikely (see below), Ca^{2+} accumulation across the Golgi/CV membrane and the affinity of cytoplasmic buffers for Sr^{2+} or Ca^{2+} may potentially influence the Sr/Ca ratio of the precipitation fluid. Ca^{2+} accumulation into the Golgi of eukaryotic cells occurs by $\text{H}^+/\text{Ca}^{2+}$ exchange driven by the (inside acidic) H^+ electrochemical gradient across the Golgi membrane which in turn is generated by V-type ATPases in eukaryotic cells (Harvey 1992). While it is very likely that such a ubiquitous transport mechanism exists in coccolithophores, its involvement in calcification is unknown. Ca^{2+} fluxes in this simple model are likely to be significantly influenced by the activity of Ca^{2+} efflux pumps both on the plasma membrane and endomembranes (Fig. 3). The slower

dynamics of changes in Sr^{2+} compared with Ca^{2+} in the cytoplasm following the activation of Ca^{2+} channels in Purkinje cell synapses has been interpreted as indicative of increased channel permeability to Sr^{2+} and reduced efficiency of Sr^{2+} extrusion from the cytosol together with lower affinity of endogenous cellular buffers for Sr^{2+} (Xu-Friedman and Regehr 1999). By analogy, Sr/Ca ratios in coccoliths are likely to be influenced by a suite of different transporters and buffers. Addressing and quantifying these problems in coccolithophores will not be easy. It must be borne in mind that experiments to investigate the physiological discrimination between Sr^{2+} and Ca^{2+} normally require Sr^{2+} concentrations that are non-physiological, i.e. much higher than those found in seawater. Full characterization of the dominant pathways delivering Ca^{2+} to the coccolith vesicle and maintaining Ca^{2+} homeostasis will require a combination of biochemical, molecular and biophysical approaches.

Approaches to Ca^{2+} transport

Application of the patch clamp technique to the coccolithophore plasma membrane may provide some insights into the major Ca^{2+} influx pathway. Our studies with the larger coccolithophore *C. pelagicus* are beginning to reveal the potential Ca^{2+} influx pathways at the plasma membrane (Fig. 4). The main ionic conductance in the plasma membrane has recently been shown to be an inwardly rectifying anion conductance that mediates Cl^- efflux (Taylor and Brownlee 2003). Its role appears to be in the maintenance and regulation of the membrane potential and it is tempting to speculate that this major conductance may be involved in charge balancing associated with HCO_3^- uptake (Taylor and Brownlee 2003). We have also identified two potential Ca^{2+} influx pathways that are undergoing further characterization (Taylor and Brownlee 2003; Fig. 4).

Ca^{2+} entering the cell across the plasma membrane may be immediately extruded by plasma membrane efflux pumps or rapidly sequestered into endomembrane compartments. Our hypothesis for the trans-cellular transport of Ca^{2+} in coccolithophores proposes that the immediate fate of the bulk of Ca^{2+} entering the cell is the endoplasmic reticulum (ER) (Berry et al. 2002). Ca^{2+} could subsequently be transported at high concentration to the CV via the normal membrane cycling processes between the ER and Golgi or even more directly to the reticular body (see Fig. 1). Indeed the ER has been shown to accumulate Ba^{2+} added as a marker for Ca^{2+} uptake in *C. pelagicus* (Berry et al. 2002). This would mean that calcification was occurring as a predominantly closed system in the coccolithophore cell. Moreover, such a mechanism would alleviate the kinetic problems associated with diffusion of sufficient Ca^{2+} across the cytosol which maintains a very low free Ca^{2+} concentration in all eukaryotes, including coccolithophores (Sanders et al. 2002; Brownlee et al. 1995). Finally, it would reduce the uphill, energy-requiring transport of Ca^{2+} into the Golgi/CV. The location of ER around the periphery of the cell and its close association with the Golgi and reticular body

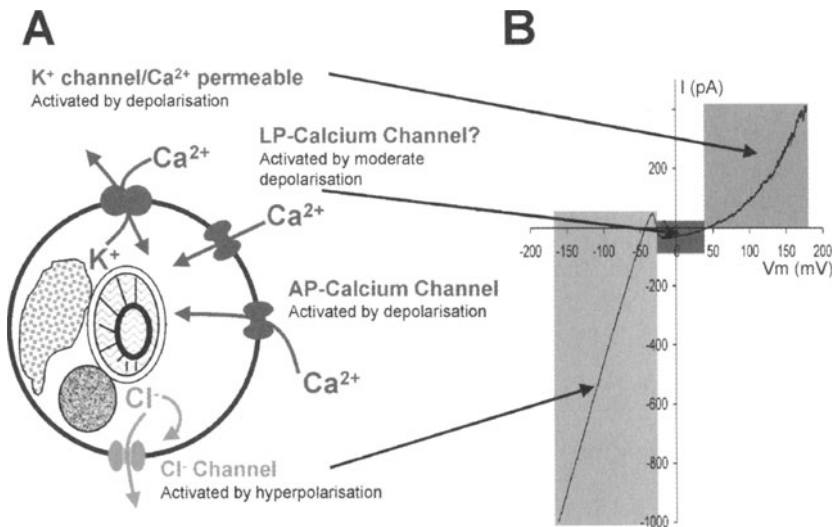


Fig. 4. Summary of the major ion channel conductances in the plasma membrane of *Coccolithus pelagicus*. **A.** Summary of ion channels characterized in patch clamp recordings illustrated in (B). Currents recorded in voltage clamp experiments, include a large Cl⁻ conductance which underlies the sensitivity of the membrane potential (V_m) to external Cl⁻. Up to three Ca²⁺-permeable cation channels have also been detected in the plasma membrane. These include K⁺-permeable channels with significant Ca²⁺ permeability. In addition, many cells exhibit a Ca²⁺-dependent action potential, which is stimulated by membrane depolarization to potentials positive of the normal resting V_m. **B.** Current passing across the plasma membrane of a *Coccolithus pelagicus* cell in response to ramping the membrane voltage from -160 mV to +180 mV. The large current passing into the cell at V_m more negative than -50 mV is carried by efflux of Cl⁻. The small inward current between -40 and +40 mV is generated by the activation of Ca²⁺-permeable cation channels, the large outward current observed at voltages above +40 mV is carried through non-selective cation channels (Taylor and Brownlee 2003).

appears to be a common feature of coccolithophores (Manton and Leedale 1969; Hori and Green 1985). Work is in progress to further elucidate the involvement of this pathway.

Concluding remarks

There are several outstanding questions relating to the mechanism and function of calcification in coccolithophores. The sources of Ci for photosynthesis and the

mechanisms of carbon transport are perhaps the most fundamental of these questions. Conflicting issues on whether calcification can, under certain conditions, facilitate photosynthesis or serve some other metabolic function also need to be resolved. Strain and species variability and likely physiological adaptation and plasticity under the wide range of conditions used in experiments may have hindered our understanding of these processes. However, it remains important that common functions and mechanisms of calcification can be shown for different strains and species. The wide range of coccolithophore strains in culture provides a potential resource to identify the differences at a molecular level that give rise to differences in physiology.

The advent of molecular and more recently the potential of genomics approaches will greatly improve the prospects of determining the roles and mechanisms of calcification. Cloning of transporter genes likely to be involved in calcification (Corstjens et al. 2001) is the first step towards their functional characterization. Future progress will depend on the development of toolkits allowing systematic knockout or mis-expression of genes of interest. The application of genomics techniques, including genome sequencing, microarrays and development of expressed sequence tag databases (ESTs) together with reporter gene technologies should provide the framework for detailed cellular studies. The recent demonstration that *E. huxleyi* can be induced to switch from haploid non-calcifying S-cell stages to diploid calcifying C-cells in culture (Laguna et al. 2001) opens opportunities for subtractive genomic or proteomic approaches applied to genetically identical calcifying or non-calcifying cells. It appears that coccolithophore cell biology is still in its infancy.

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Pigment diversity of coccolithophores in relation to taxonomy, phylogeny and ecological preferences

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Summary

Pigment analyses can be employed to study distribution, abundance and composition of natural phytoplankton populations using a chemotaxonomic approach. Cultured haptophytes have played a relevant role in advances in this analytical field and many pigments described in the literature were first detected in members of this algal group. The present chapter provides an historical overview of pigment detection in the Haptophyta and contains new data on their distribution in cultured coccolithophores (some of which not previously cultured), analysed in the context of the EC funded CODENET project. This comparative HPLC study was conducted with the largest (73 monoclonal strains) and most diverse (36 species representing ten families and four orders, including six holococcolithophores) haptophyte sample set ever subjected to a comparative study. The observed extraordinary diversity in the pigment composition (based on ten carotenoids, five polar- and three non-polar chlorophyll types) appeared closely related to current taxonomy and published phylogeny. Chl *a* and the accessory pigments MgDVP, Chl *c*₂, Ddx, Dtx and β,β -carotene comprised the common haptophyte pigment load. Based on ecological preferences, all species synthesised either Chl *c*₁ (littoral, benthic or brackish waters) or HfX (open-ocean and non-littoral coastal environments). HfX thus recovered its previously questioned status as a straightforward marker for haptophyte distribution in offshore habitats. Haploid and diploid life-cycle stages (analysed separately for the first time in both *Coccolithus* and *Emiliana*) yielded identical pigment compositions. This provided further evidence for

an evolutionary origin of pigment content, rather than short-term temporal adaptations to environmental conditions.

Introduction

Phytoplankton form the base of nearly all marine food webs. Therefore, a detailed study of their abundance (total biomass), structure (taxonomic composition, size-class distribution) and function (carbon fixation rate, new and regenerated production) is central for understanding the basics of marine ecology and global elemental cycles.

The phytoplankton division Haptophyta is one of the most abundant and geographically widespread components of natural phytoplankton assemblages. It includes the coccolithophores, which form the focus of the present book. Coccolithophores are interesting from several points of view and have been the subject of extensive multidisciplinary studies discussed in the other chapters of this volume. However, it should be noted that these beautiful calcareous organisms are only one of the many taxonomic components comprised in natural phytoplankton communities. Comparative studies of coccolithophore ecology and abundance should, therefore, address their relative importance in the field in relation to the other (non-calcifying) haptophytes and to total phytoplankton biomass.

Distribution patterns of coccolithophores and other robust algae (like silica-walled diatoms and armoured dinoflagellates) are commonly evaluated using light and/or electron microscopy. However, these techniques are not equally well suited for most other taxonomic groups. Many phytoplankton species are delicate and are often damaged beyond recognition during sampling and fixation, whereas others are simply too small or may lack useful morphological features for microscopic identification at all times (Gieskes and Kraay 1983a; Murphy and Haugen 1985; Hooks et al. 1988). These detection and identification problems also affect quantification of coccolithophore biomass, because the lightly- or non-calcifying life-cycle stages of many species are overlooked. Such cells, and those of all other phytoplankton species, can be detected using a flow cytometer, but at present these sophisticated laser-operated instruments provide little taxonomic information on the eukaryotic fraction (Carr et al. 1996).

Alternative approaches such as cloning and sequencing of 18S rRNA eukaryotic genes have been very useful providing taxonomic information on (pico)eukaryotic assemblages (Díez et al. 2001a; López-García et al. 2001; Moon-Van der Staay et al. 2001), but are very time consuming and thus not suitable for routine observations. The molecular fingerprinting techniques T-RFLP (terminal restriction fragment length polymorphism) (Liu et al. 1997; Marsh 1999; Moesender et al. 1999) and DGGE (denaturing gradient gel electrophoresis) (Muyzer et al. 1997; Muyzer 1999; Díez et al. 2001b) are quicker and currently the best compromise between the number of samples processed and the information obtained. However, the main question of these techniques as applied to marine stud-

ies is whether the extraction of DNA and/or the PCR steps (necessary preliminary procedures) induce biases providing a false image regarding the composition of the natural phytoplankton assemblages (Suzuki et al. 1998).

The pigment approach

Chemotaxonomic techniques based on analyses of photosynthetic pigments (reviewed by Jeffrey et al. 1997b) have been developed as a complementary tool, providing a possible solution to limitations associated with the microscopic and flow cytometric approaches. Haptophytes (coccolith-bearing and non-calcifying species) have played an important role in advances in this analytical field and have been a continuous source of new chlorophylls and carotenoids described in the literature. An overview is provided in Table 1.

All phytoplankton species and other oxygenic photosynthetic organisms synthesize a monovinyl (MV; eukaryotes and *Synechococcus* type prokaryotes) or a divinyl (DV; *Prochlorococcus* type prokaryotes only) form of Chl *a*. The sum of these two compounds (total Chl *a*; TChl *a*) is easily quantified *in vivo* using a fluorometer attached to a CTD (e.g. vertical profiles) or, more accurately, *in vitro* (e.g. samples collected from discrete depths of interest), using acetone extracts

Table 1. Overview of haptophyte chlorophylls and carotenoids mentioned in the text, their common abbreviations and codes of those considered in Fig. 1.

Nr.	Pigment	Abbreviation	Codes
01	Divinyl chlorophyll <i>c</i> <i>P. gyraus</i> -type	DV-Chl c_{PAV}	-
02	Monovinyl chlorophyll <i>c</i> <i>E. gayraliae</i> -type	MV-Chl c_{PAV}	-
03	Divinyl chlorophyll c_3	DV-Chl c_3 or Chl c_3	DV c_3
04	Monovinyl chlorophyll c_3	MV-Chl c_3	MV c_3
05	Divinyl Protochlorophyllide	MgDVP	-
06	(Divinyl) Chlorophyll c_2	Chl c_2	-
07	(Monovinyl) Chlorophyll c_1	Chl c_1	c_1
08	Chl c_2 -monogalactosyldiacylglyceride <i>E. hux</i> -type	Chl c_2 -MGDG _{Ehux}	c_2E
09	Chlorophyll <i>a</i>	Chl <i>a</i>	-
10	Chl c_2 -monogalactosyldiacylglyceride <i>C. pol</i> -type	Chl c_2 -MGDG _{Cpoly}	c_2C
11	Fucoxanthin	Fx	Fx
12	Unknown Fucoxanthin-like	Unk-Fx	FxL
13	4-keto-Fucoxanthin	4kFx	4kFx
14	19'-Butanoyloxyfucoxanthin	BFx	BFx
15	19'-Hexanoyloxyfucoxanthin	HFx	HFx
16	4-keto-Hexanoyloxyfucoxanthin	4kHFx	4kHFx
17	Diadinoxanthin	Ddx	-
18	Diatoxanthin	Dtx	-
19	β,ϵ -Carotene	α -Carotene	$\beta\epsilon$
20	β,β -Carotene	β -Carotene	-

analyzed in a bench fluorometer (Holm-Hansen et al. 1965; Welschmeyer 1994).

Chl *a* is thus commonly employed as a practical proxy for total phytoplankton biomass. This approach is now widely used by satellite imagery, allowing studies of phytoplankton distribution at a global scale (synoptic observations), as well as continuous monitoring of regions of specific interest (e.g. seasonal variations for management of fisheries) and remote areas difficult to access by other means. However, results obtained from such ocean color registrations are restricted to the shallower layers of the water column, whereas phytoplankton accumulates in a deep chlorophyll maximum (DCM) for most of the year as a consequence of water column stratification. Remote sensing cannot therefore fully substitute fieldwork, although it is a very practical way to detect areas worth attending for ship-born measurements.

In addition to MV or DV-Chl *a*, all photosynthetic organisms synthesise a range of accessory chlorophyll types and carotenoids. Some of these compounds are directly active in photosynthesis through an efficient light energy transfer to Chl *a* (Falkowski and Raven 1997), whereas others have a photoprotective function through interaction in photochemically induced oxidation reactions at high irradiance conditions (e.g. carotenoids implied in the xanthophyll cycle; Demers et al. 1991; Long and Humphries 1994; Lohr and Wilhelm 1999).

Terrestrial plants only contain a few different accessory pigments and their compositions are boringly similar. Phytoplankton, on the other hand, synthesise a wide range of different carotene and chlorophyll types with an extraordinary variation between species. Each of these compounds has a characteristic absorbance spectrum and specific absorbance maxima (signatures used for identification; see Jeffrey et al. 1997a). It is generally assumed that the complex and variable pigment composition of phytoplankton reflects adaptations to life in a 3-dimensional aquatic environment, where spectral composition and quantity of photosynthetically active radiation (PAR) is progressively modified by the water column (Kirk 1994). However, there are seemingly many different solutions to the same light-harvesting problem, as evidenced by the fact that pigment composition varies greatly among species sharing the same ecological niche (for example the DCM). On the other hand, similarities in pigment composition do generally increase towards the lower taxonomic levels (Jeffrey and Vesk 1997), even if the taxa occupy rather different niches. This indicates that pigment patterns have a strong evolutionary background (i.e. metabolic processes required for their synthesis are genetically coded), rather than reflecting temporal adaptations of individuals to specific ecological conditions.

Pigments can hence be used as practical chemotaxonomic markers for phytoplankton studies in natural ecosystems, allowing rapid estimation of the relative abundances of different phytoplankton groups (Everitt et al. 1990; Letelier et al. 1993; Andersen et al. 1996), and are of potential use for phylogenetic reconstructions (Delwiche and Palmer 1997). Both applications require detailed knowledge of pigment distribution in cultured species and implementation of suitable analytical procedures, able to detect all pigments synthesized by representatives of the taxonomic group(s) of interest.

Pigment analytical procedures

A range of different methods have been proposed to estimate concentrations of carotenoids and chlorophyll types after extraction in organic solvents (usually acetone or methanol). Early approaches relied on differences in absorbance (Richards and Thomson 1952; Lorenzen 1967; Jeffrey and Humphrey 1975) or fluorescence properties of pigments (Loftus and Carpenter 1971). These procedures could only assess a limited number of compounds and interference of signals from different pigments could produce unreliable results (see review by Mantoura et al. 1997).

The best way to measure concentrations of pigments is to separate (physically) the natural mixture into its individual components, followed by identification and quantification steps (i.e. 'chromatography'). Very good pigment separations were achieved using paper chromatography (Jensen and Sakshaug 1973) and thin-layer chromatography (Jeffrey 1974, 1981), but since the mid-1980s analyses have mainly relied on more efficient high-performance liquid chromatography (HPLC) methods.

Simultaneous separation of all pigments associated with natural phytoplankton communities or unialgal cultures is not an easy task, because these compounds cover a wide range of polarities and many share almost identical chemical structures (see Jeffrey et al. 1997a). Especially difficult is the analysis of MV/DV chlorophyll pairs, now known to occur for nearly all chlorophyll types described in the literature.

Solving analytical limitations has been a challenge for chromatographic method development and many different protocols have been published over the years (reviewed by Garrido and Zapata in press). Most methods currently employed for routine pigment analyses are based on reversed-phase (RP) HPLC procedures using a thermostated monomeric or a polymeric C₈ or C₁₈ column and binary gradient elution. Normal-phase (NP) HPLC is not employed, because this approach causes severe degradation of the polar Chl *c* pigments (Gieskes and Kraay 1983b).

Parallel to advances in the chromatographic field the number of pigments described for phytoplankton has continuously increased. Not a single HPLC method currently available achieves a simultaneous separation of all pigments commonly distributed in oceanic environments, which underlines the difficulty inherent in this analytical field. Since choice of method necessarily involves a compromise, and implementation a time-consuming period of adaptation, the character of the sample(s) to be analysed and the specific scientific objectives should be carefully considered.

Overview of haptophyte pigments

Literature on chromatographic method development has frequently involved the haptophyte *Emiliania huxleyi* (Lohmann) Hay et Mohler, classified in the class Prymnesiophyceae Hibberd. As a consequence many new pigments described in

the literature were first isolated from cultured strains of this ubiquitous coccolithophore.

Initial analysis of *E. huxleyi* revealed the presence of the carotenoids diadinoxanthin (Ddx), diatoxanthin (Dtx), two fucoxanthin (Fx) fractions, β,β -carotene and chlorophyll *c* (Jeffrey and Allen 1964). Improved methods (Jeffrey 1969, 1972) enabled separation of Chl *c* (previously thought to be a single, polar, compound) into two different pigments, referred to as Chl *c*₁ and Chl *c*₂ (based on elution order with the chromatographic procedures employed). These two Chl *c* forms (*E. huxleyi* only contains the latter) were identified as MV (Dougherty et al. 1970) and DV (Budzikiewicz and Taraz 1971) analogues, respectively. The indications 'Chl *c*' or 'Chl *c*₁+*c*₂' are currently employed for data obtained with procedures that do not allow separation of the MV/DV analogues.

Further studies of *E. huxleyi* revealed the presence of β,ϵ -carotene (frequently indicated as ' α -carotene') and an unidentified, though important, carotenoid (Norgård et al. 1974). The latter was identified as the Fx derivative 19'-hexanoyloxyfucoxanthin (HFX) by Arpin et al. (1976).

HFX was initially assumed to be typical for all haptophyte species and hence employed as a 'marker pigment' for biomass estimates (expressed in terms of associated Chl *a*) of this algal group (Everitt et al. 1990; Letelier et al. 1993; Andersen et al. 1996). This single marker pigment approach (also employed for other taxonomic groups using different carotenoids) was later questioned when comparative analysis of haptophyte cultures revealed HFX in only a restricted number of species and defined pigment types (Jeffrey and Wright 1994; Garrido 1997; Rodríguez 2001).

Jeffrey and Wright (1987) detected the presence of a third polar Chl *c* type pigment (thus referred to as Chl *c*₃) in cultured *E. huxleyi*, identified as a DV compound (Fookes and Jeffrey 1989). More selective HPLC procedures showed that *E. huxleyi* also contained a second, spectrally different, Chl *c*₃ form (Garrido and Zapata 1993), identified as a novel MV pigment (Garrido et al. 1995; Garrido and Zapata 1998).

Garrido and Zapata (1998) discovered the presence of an additional, possibly HFX related, novel carotenoid. This novel compound was only much later identified as 4-keto-HFX (Egeland et al. 2000). The previously described 4-keto-Fx (Egeland et al. 1999) was detected in the coccolithophores *Ochrosphaera verrucosa* Schussnig and *Ochrosphaera neapolitana* Schussnig, but not in *E. huxleyi* (Rodríguez 2001).

Fawley (1989) detected a fourth polar Chl *c* type (although never indicated as 'Chl *c*₄') in the non-calcifying haptophyte *Pavlova gyrans* Butcher emend. Green et Manton, classified in the class Pavlovophyceae (Cavalier-Smith) Green et Medlin. This pigment (frequently indicated as 'Chl *c*-*P. gyrans*') has never been mentioned to occur in any member of the class Prymnesiophyceae, but was detected in Pseudo-nitzschia Hassel type diatoms (Zapata et al. 2000a).

During a comparative study of the class Pavlovophyceae a second form of Chl *c*-*P. gyrans*, not previously described in the literature, was detected in a cultured strain of *Exanthemachrysis gayraliae* Lepailleur (Van Lenning et al. 2003). Based

on differences in spectral properties and chromatographic behavior the authors identified the new and previously detected forms as MV (Chl c_1 -like) and DV (Chl c_2 -like) analogues, respectively, although their molecular structure remains unknown. Observed distribution of these two chlorophyll forms, for practical reasons referred to as MV and DV-Chl c_{PAV} (therewith respecting their taxonomic origin of detection) among the species studied appeared perfectly related to phylogenetic results. MV-Chl c_{PAV} was recently also mentioned to occur in the dinoflagellate *Kryptoperidinium foliaceum* (Stein) Lindemann (Zapata et al. in press), but whether this indicates a close genetic relationship to *E. gayraliae* or possibly reflects the endosymbiotic origin of the chloroplasts of this dinoflagellate remains to be confirmed.

The last polar Chl c -like compound detected in the Prymnesiophyceae was MgDVP (Garrido et al. 1995), first described for flagellates classified in the division Prasinophyta Möhn (Ricketts 1966). MgDVP was identified as a precursor in the synthesis of Chls a , c_1 or c_2 (Porra et al. 1997) and hence its presence can be expected in extracts of any algal species. The late discovery of this compound in haptophytes is due to the extreme analytical difficulty of separating this pigment from Chls c_1 and/or c_2 , both of which are not present in prasinophytes.

MgDVP and the other Chl c types mentioned above are polar pigments, and thus among the first compounds detected (i.e. short retention times) when employing RP-HPLC. Phytoplankton may, however, also synthesize non-polar Chl c forms (characterized by longer retention times) eluting towards the end of the analysis, near (or coeluting with) Chl a .

First published records on a non-polar Chl c form were based on field samples collected during a bloom of the non-calcifying haptophyte *Corymbellus aureus* Green (Gieskes and Kraay 1986) and cultured *E. huxleyi* (Nelson and Wakeham 1989). The non-polar character of this Chl c_2 -like pigment was attributed to its link with the plant membrane lipid monogalactosyldiacylglyceride (MGDG) (Garrido et al. 2000) containing two fatty acid residues.

Zapata et al. (2001) identified the structure of a second non-polar Chl c_2 type, first detected in the non-calcifying haptophyte *Chrysochromulina polylepis* Mantón et Parke (Zapata et al. 1998). This compound only differed from the former in the absence of one of the attached fatty acid residues and other combinations were expected to occur. Zapata et al. (2001) confirmed this possibility by detecting the presence of at least five further non-polar Chl c_2 types in addition to Chl c_2 -MGDG *E. huxleyi*-type and Chl c_2 -MGDG *C. polylepis*-type. The molecular structures of these compounds remain, however, to be elucidated.

It is likely that many of the non-polar Chl c_2 types observed have MV (Chl c_1 -like) counterparts, but until now only one of them has been described for the non-calcifying haptophyte *Prymnesium parvum* Carter (Garrido et al. 1995). Non-polar Chl c_3 types, on the other hand, have not yet been reported for any species.

Distribution of pigments among coccolithophores

Knowledge on the distribution of the currently known haptophyte carotenoids, polar and non-polar Chl *c* pigments (summarized in Table 1) among coccolithophores is limited. The number of species available in culture collections is extremely small compared to the diversity observed in the field, and not all families are represented in culture. In the context of the CODENET project special attention was therefore given to isolation and culture of additional species covering the diversity of coccolithophores.

Successful new isolates and a range of additional coccolithophore species, kindly provided by the ALGOBANK culture collection in Caen <http://www.unicaen.fr/algoBank> or obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP), were submitted to selective HPLC pigment analyses. Five members of the non-calcifying haptophyte family Isochrysidaceae Parke were included, due to their close taxonomic and genetic (Fujiwara et al. 2001) relationship with the coccolith-bearing family Noelaerhabdaceae Jerkovic emend. Young et Bown.

Determined variations in pigment patterns were compared with current taxonomy (based on Cros and Fortuño 2002, with modifications of Sáez et al. this volume), published molecular phylogeny (Fujiwara et al. 2001), molecular clock calculations (Medlin et al. 1997; Sáez et al. 2003) and known ecological preferences. These comparisons revealed several clear-cut (evolutionary) patterns. Relative proportions of pigments were not considered (although trace-level compounds were indicated), but it is well-known that they may vary according to irradiance conditions, nutrient status, age and physiological state of the culture.

Methods

All 73 monoclonal haptophyte strains (36 species, including six holococcolithophores), representing ten families and four orders (details summarized in Table 2), were grown in filter-sterilized K/5 enriched seawater medium (Keller et al. 1987) at 17°C using artificial light sources ($80 \mu\text{Einstein m}^{-2} \text{ s}^{-1}$) in a 14:10 hour light:dark cycle. Algal cells were harvested during the logarithmic phase of growth and concentrated by gentle vacuum filtration onto glass-fiber filters (25 mm Whatman GF/F). Taxonomic identity was confirmed by scanning or transmission electron microscopy, and culture purity verified under high magnification in a light microscope. Haploid and diploid life-cycle stages of one strain each of *Emiliania huxleyi* and *Coccolithus pelagicus* (Wallich) Schiller were sampled separately. Filters were blotted on absorbent paper until no humidity was observed on the latter and stored frozen (-80°C) until extraction. Pigments were extracted in 90% HPLC-grade acetone to avoid the risk of pigment degradation and losses inevitably associated with methanol (Latasa et al. 2001). Pigment analyses were performed using the method described by Zapata et al. (2000b). This method was se-

lected due to its excellent selectivity towards pigments described for the Prymnesiophyceae and it is probably also the most practical compromise available to date for routine analysis of natural field samples. Extraction procedures, adaptations of the solvent gradient and details on the chromatographic instrument (Thermo Separation) were the same as previously described (Van Lenning et al. 2003). The column employed (Waters Symmetry C₈, 150 x 4.6 mm i.d., 3 µm particle size, protected with a guard-column containing the same stationary phase) was thermostated at 25°C using a re-circulating water bath. Retention times and absorbance characteristics of peaks detected were compared with those of authentic standards (employed for calibration procedures of the equipment) obtained from DHI (Denmark), and results obtained for species with well-known pigment compositions (e.g. *Emiliana huxleyi* and *Pavlova gyrams*).

Table 2. Culture strains used in this study. All strains are currently maintained in the Algaebank-Caen culture collection at the University of Caen, France. For further information on strains see <http://www.unicaen.fr/algaebank> and <http://ccmp.bigelow.org/>

Species, strain	Origin, isolator, data
<i>Pleurochrysis roscoffensis</i> HAP32	Brittany, France, J. Fresnel, 1976
<i>Pleurochrysis pseudoroscoffensis</i> HAP48	Normandy, France, J. Fresnel, 1979
<i>Pleurochrysis gayraliae</i> HAP10	CCAP912/1, E. Pringsheim, pre1952
<i>Pleurochrysis carterae</i> HAP1	N. Atlantic, Morocco, J. Fresnel, 1983
<i>Pleurochrysis elongata</i> HAP86	CCAP961/1, M. Parke, 1949
<i>Pleurochrysis scherffellii</i> HAP11	Normandy, France, H. Lepailleur, 1965
<i>Pleurochrysis placolithoides</i> HAP59	Brittany, France, J. Fresnel, 1983
<i>Pleurochrysis carterae</i> var. <i>dentata</i> HAP6	California, USA, R. Lewin, 1989
<i>Jomonolithus littoralis</i> AC513	Mediterranean, Spain, J. Fresnel, 1999
<i>Hymenomonas globosa</i> HAP30	Normandy, France, C. Billard, 1971
<i>Hymenomonas coronata</i> HAP58	Sarasota, USA, J. Fresnel, 1983
<i>Ochrosphaera verrucosa</i> HAP82	PLY466
<i>Ochrosphaera neapolitana</i> HAP12	Normandy, France, H. Lepailleur, 1965
<i>Cruciplacolithus neohelis</i> HAP39	Mediterranean, Syria, J. Fresnel, 1972
<i>Coccolithus pelagicus</i> (large) KL2	N. Atlantic, France, I. Probert, 1999
<i>Coccolithus pelagicus</i> (large) CC32	N. Atlantic, Portugal, I. Probert, 1999
<i>Coccolithus pelagicus</i> (large) CC35	N. Atlantic, Portugal, I. Probert, 1999
<i>Coccolithus pelagicus</i> (large) N476-2	S. Atlantic, Namibia, I. Probert, 2000
<i>Coccolithus pelagicus</i> (large) AS56T	Mediterranean, Spain, I. Probert, 1999
<i>Coccolithus pelagicus</i> (small) IBV73	N. Atlantic, Iceland, I. Probert, 1999
<i>Calyptrorphaera radiata</i> NIESP80	NIESP80
<i>Calyptrorphaera sphaeroidea</i> UTEXLB1940	UTEXLB1940
<i>Calyptrorphaera</i> sp. NIESCaly1	NIESCaly1
<i>Calyptrorphaera</i> sp. NIESCaly2	NIESCaly2
<i>Helladosphaera</i> sp. NIESHe	NIESHe
<i>Umbilicosphaera hultburiana</i> NS3-A	S. Atlantic, S. Africa, I. Probert, 1999
<i>U. sibogae</i> var. <i>sibogae</i> ASM39	Mediterranean, Spain, M. Geisen, 1999

Table 2. (cont.)

<i>Umbilicosphaera sib.</i> var. <i>foliosa</i> ESP6M1	Mediterranean, Spain, I. Probert, 1999
<i>Oolithotus fragilis</i> AS641	Mediterranean, Spain, I. Probert, 1999
<i>Oolithotus fragilis</i> AS553	Mediterranean, Spain, I. Probert, 1999
<i>Calcidiscus leptoporus</i> ASM25	Mediterranean, Spain, I. Probert, 1999
<i>Calcidiscus leptoporus</i> ASM31	Mediterranean, Spain, I. Probert, 1999
<i>Calcidiscus leptoporus</i> PC13	N. Atlantic, Ireland, I. Probert, 1998
<i>Helicosphaera carteri</i> var. <i>carteri</i> NS10B	S. Atlantic, S. Africa, I. Probert, 1999
<i>Helicosphaera carteri</i> var. <i>carteri</i> NS8-5	S. Atlantic, S. Africa, I. Probert, 1999
<i>Helicosphaera carteri</i> var. <i>hyalina</i> NAP11	Mediterranean, Italy, I. Probert, 2000
<i>Scyphosphaera apsteinii</i> TW19	Mediterranean, Spain, I. Probert, 2001
<i>Algirosphaera robusta</i> ASM24	Mediterranean, Spain, I. Probert, 1999
<i>Syracosphaera pulchra</i> GK3	Mediterranean, Spain, I. Probert, 2000
<i>Syracosphaera pulchra</i> GK17	Mediterranean, Spain, I. Probert, 2000
<i>Syracosphaera pulchra</i> GK15	Mediterranean, Spain, I. Probert, 2000
<i>Syracosphaera pulchra</i> NAP1	Mediterranean, Italy, I. Probert, 2000
<i>Coronosphaera mediterranea</i> NS8-6	S. Atlantic, S. Africa, I. Probert, 1999
<i>Emiliana huxleyi</i> AS56B	Mediterranean, Spain, I. Probert, 1999
<i>Emiliana huxleyi</i> ESP410	Mediterranean, Spain, I. Probert, 1998
<i>Emiliana huxleyi</i> BP91	N. Atlantic, Iceland, I. Probert, 1999
<i>Emiliana huxleyi</i> JS2	Mediterranean, Tunisia, I. Probert, 1998
<i>Emiliana huxleyi</i> CCMP370	N. Atlantic, Oslo Fjord, E. Paasche, 1959
<i>Emiliana huxleyi</i> CCMP371	N. Atlantic, Sargasso, B. Palenik, 1987
<i>Emiliana huxleyi</i> CCMP372	N. Atlantic, Sargasso, B. Palenik, 1987
<i>Emiliana huxleyi</i> CCMP373	N. Atlantic, Sargasso R. Guillard, 1960
<i>Emiliana huxleyi</i> CCMP374	N. Atl., Gulf of Maine, T. Skinner, 1989
<i>Emiliana huxleyi</i> CCMP375	N. Atlantic, Bermuda, R. Guillard, 1987
<i>Emiliana huxleyi</i> CCMP376	N. Atl., Gulf of Maine, M. Keller, 1986
<i>Emiliana huxleyi</i> CCMP377	N. Atl., Gulf of Maine, R. Selvin, 1988
<i>Emiliana huxleyi</i> CCMP378	N. Atl., Gulf of Maine, R. Selvin, 1988
<i>Emiliana huxleyi</i> CCMP379	M. Parke
<i>Emiliana huxleyi</i> CCMP1280	S. Atlantic, Brazil, F. Valios, 1985
<i>Emiliana huxleyi</i> CCMP1516	S. Pacific, S. America, D. Jacobson, 1991
<i>Emiliana huxleyi</i> CCMP1742	Open Pacific, R. Waters, pre1996
<i>Emiliana huxleyi</i> (type R) TQ24	Tasman Sea, NZ, I. Probert, 1998
<i>Emiliana huxleyi</i> (type R) TQ6	Tasman Sea, NZ, I. Probert, 1998
<i>Emiliana huxleyi</i> (type R) TQ25	Tasman Sea, NZ, I. Probert, 1998
<i>Emiliana huxleyi</i> (type R) TQ26	Tasman Sea, NZ, I. Probert, 1998
<i>Gephyrocapsa oceanica</i> JS14	Mediterranean, Tunisia, I. Probert, 1998
<i>Gephyrocapsa oceanica</i> AS62E	Mediterranean, Spain, I. Probert, 1999
<i>Gephyrocapsa oceanica</i> ESP182	Mediterranean, Spain, I. Probert, 1998
<i>Gephyrocapsa oceanica</i> ESP6CL4	Mediterranean, Spain, I. Probert, 1999
<i>Isochrysis litoralis</i> HAP18	Normandy, France, H. Lepaillieur, 1965
<i>Isochrysis galbana</i> HAP34	PLY, 1973
<i>Pseudoisochrysis paradoxa</i> CCAP949/1	CCAP949/1, F. Ott, 1974
<i>Chrysotila lamellosa</i> HAP17	Normandy, France, C. Billard, 1968
<i>Dicrateria</i> sp. HAP49	N. Atlantic, Morocco, J. Fresnel, 1986

Orders / Families	Nr. / Species	Strains	Carotenoids	Chlorophylls
Coccolith-bearing Haptophyta (Class Prymnesiophyceae)	Pleurochrysidaceae	1 <i>Jomonolithus littoralis</i>	AC513 Fx,	Chl c_1 ,
		2 <i>Pleurochrysis roscoffensis</i>	HAP32 Fx,	Chl c_1 ,
		3 <i>Pleurochrysis pseudoroscoff.</i>	HAP48 Fx,	Chl c_1 ,
		4 <i>Pleurochrysis elongata</i>	HAP86 Fx,	Chl c_1 ,
		5 <i>Pleurochrysis carterae</i>	HAP1 Fx,	Chl c_1 ,
		6 <i>Pleurochrysis placolithoides</i>	HAP59 Fx,	Chl c_1 ,
		7 <i>Pleurochrysis scherffellii</i>	HAP11 Fx,	Chl c_1 ,
		8 <i>Pleurochrysis gayraliae</i>	HAP10 Fx,	*4kFx/BFx, Chl c_1 ,
		9 <i>Pleurochrysis carterae dentata</i>	HAP6 Fx,	*4kFx/BFx, Chl c_1 ,
	Hymenomonadaceae	10 <i>Hymenomonas globosa</i>	HAP30 Fx,	*4kFx/BFx, Chl c_1 ,
		11 <i>Hymenomonas coronata</i>	HAP58 Fx,	*4kFx/BFx, Chl c_1 ,
		12 <i>Ochrosphaera neapolitana</i>	HAP12 Fx,	4kFx/BFx, Chl c_1 , DV c_3 ,
		13 <i>Ochrosphaera verrucosa</i>	HAP82 Fx,	4kFx/BFx, Chl c_1 , DV c_3 ,
	Coccolithaceae	14 <i>Cruciplacolithus neohelis</i>	HAP39 Fx, β e,	*4kFx/BFx, Chl c_1 , DV c_3 ,
		15 <i>Coccolithus pelagicus</i> (large)	CC32 Fx, β e, HfX, 4kHfX,	4kFx/BFx, Chl c_1 , DV c_3 , *MV c_3 , c_2E , c_2C
		16 <i>Coccolithus pelagicus</i> (large)	CC35 Fx, β e, HfX, 4kHfX,	4kFx/BFx, Chl c_1 , DV c_3 , *MV c_3 , c_2E , c_2C
		17 <i>Coccolithus pelagicus</i> (large)	N476-2 Fx, β e, HfX, 4kHfX,	4kFx/BFx, Chl c_1 , DV c_3 , *MV c_3 , c_2E , c_2C
		18 <i>Coccolithus pelagicus</i> (large)	AS56T Fx, β e, HfX, 4kHfX, *FxL,	4kFx/BFx, Chl c_1 , DV c_3 , *MV c_3 , c_2E , c_2C
		19 <i>Coccolithus pelagicus</i> (large)	KL2 Fx, β e, HfX, 4kHfX, *FxL,	4kFx/BFx, Chl c_1 , DV c_3 , *MV c_3 , c_2E , c_2C
		20 " (flagellated stage)	KL2 Fx, β e, HfX, 4kHfX, *FxL,	4kFx/BFx, Chl c_1 , DV c_3 , *MV c_3 , c_2E , c_2C
		21 <i>Coccolithus pelagicus</i> (small)	IBV73 Fx,	HfX, 4kHfX, *FxL, 4kFx/BFx,
		22 <i>Calyptrosphaera radiata</i>	NIESP80 Fx,	HfX, 4kHfX, *FxL, 4kFx/BFx,
		23 <i>Calyptrosphaera radiata</i>	UTEXLB1940 Fx,	HfX, 4kHfX, *FxL, 4kFx/BFx,
		24 <i>Calyptrosphaera sp.</i>	NIESCALY1 Fx,	HfX, 4kHfX, *FxL, 4kFx/BFx,
		25 <i>Calyptrosphaera sp.</i>	NIESCALY2 Fx,	HfX, 4kHfX, *FxL, 4kFx/BFx,
		26 <i>Helladosphaera sp.</i>	NIESHe Fx,	HfX, 4kHfX, *FxL, 4kFx/BFx,
	Holococcoliths	27 <i>Umbilicosphaera sib. sibogae</i>	ASM39 *Fx, β e, HfX,	*FxL,
		28 <i>Umbilicosphaera sib. foliosa</i>	ESP6M1 *Fx, β e, HfX,	*FxL,
		29 <i>Umbilicosphaera hulbertiana</i>	NS3-A *Fx, β e, HfX,	*FxL,
		30 <i>Oolithus fragilis</i>	AS641 *Fx, β e, HfX,	*FxL, 4kFx/BFx,
		31 <i>Oolithus fragilis</i>	AS553 *Fx, β e, HfX,	*FxL, 4kFx/BFx,
		32 <i>Calcidiscus leptoporus</i>	ASM25 β e, HfX,	*FxL, 4kFx/BFx,
		33 <i>Calcidiscus leptoporus</i>	ASM31 β e, HfX,	*FxL, 4kFx/BFx,
		34 <i>Calcidiscus leptoporus</i>	PC13 β e, HfX,	*FxL, 4kFx/BFx,
		35 <i>Helicosphaera cart. var. carteri</i>	NS10B *Fx, β e, HfX,	*FxL,
		36 <i>Helicosphaera cart. var. carteri</i>	NS8-5 *Fx, β e, HfX,	*FxL,
	Zygodiscales	37 <i>Helicosphaera cart. var. hyalina</i>	NAP11 Fx, β e, HfX, 4kHfX,	*FxL,
		38 <i>Scyphosphaera apsteinii</i>	TW19 HfX,	*FxL,
		39 <i>Algirosphaera robusta</i>	ASM24 *Fx,	HfX, 4kHfX, *FxL,
	Syracosphaerales	40 <i>Coronosphaera mediterranea</i>	NS8-6 *Fx, β e, HfX, 4kHfX,	*FxL,
		41 <i>Syracosphaera pulchra</i>	GK17 β e, HfX,	
		42 <i>Syracosphaera pulchra</i>	GK15 β e, HfX,	
		43 <i>Syracosphaera pulchra</i>	NAP1 β e, HfX,	
		44 <i>Syracosphaera pulchra</i>	GK3 β e, HfX,	
	Noelaerhabdaceae	45 <i>Emiliania huxleyi</i>	AS56B Fx, β e, HfX, 4kHfX,	FxL,
		46 <i>Emiliania huxleyi</i>	ESP410 Fx, β e, HfX, 4kHfX,	FxL,
		47 <i>Emiliania huxleyi</i>	BP91 Fx, β e, HfX, 4kHfX,	FxL,
		48 <i>Emiliania huxleyi</i>	JS2 Fx, β e, HfX, 4kHfX,	FxL,
		49 <i>Emiliania huxleyi</i> (type R)	TQ26 Fx, β e, HfX, 4kHfX,	FxL,
		50 " (flagellated stage)	TQ26 Fx, β e, HfX, 4kHfX,	FxL,
		51 <i>Emiliania huxleyi</i> (type R)	TQ25 Fx,	HfX, 4kHfX, FxL,
		52 <i>Emiliania huxleyi</i> (type R)	TQ24 Fx,	HfX, 4kHfX, FxL,
		53 <i>Emiliania huxleyi</i> (type R)	TQ06 Fx,	HfX, 4kHfX, FxL,
		54 <i>Gephyrocapsa oceanica</i>	JS14 Fx,	HfX, 4kHfX, FxL,
		55 <i>Gephyrocapsa oceanica</i>	AS62E Fx,	HfX, 4kHfX, FxL,
		56 <i>Gephyrocapsa oceanica</i>	ESP182 Fx,	HfX, 4kHfX, FxL,
		57 <i>Gephyrocapsa oceanica</i>	ESP6C14 Fx,	HfX, 4kHfX, FxL,
	Isochrysidales	58 <i>Isochrysis littoralis</i>	HAP18 Fx,	Chl c_1 , DV c_3 ,
		59 <i>Isochrysis galbana</i>	HAP34 Fx,	Chl c_1 ,
		60 <i>Pseudoisochrysis paradoxa</i>	CCAP949/1 Fx,	Chl c_1 ,
		61 <i>Chrysothila lamellosa</i>	HAP17 Fx,	Chl c_1 ,
		62 <i>Dicrateria sp.</i>	HAP49 Fx,	Chl c_1 ,

Fig. 1. Current taxonomic classification of the haptophytes studied and the associated accessory pigments. The common pigments MgDVP, Chl c_2 , Ddx, Dtx, β , β -carotene were omitted from these comparative results.

Results and Discussion

HPLC analyses revealed the presence of all pigments previously described for the class Prymnesiophyceae, but compositions showed marked differences at the taxonomic level. The accessory pigments MgDVP, Chl c_2 , Ddx, Dtx and β , β -carotene were found in all strains analyzed and therewith classified as the basic haptophyte pigment load. These compounds were omitted from comparative results (Fig. 1). The carotenoid Fx, generally considered to be a common haptophyte pigment, was maintained, because concentrations remained undetectable or very low in 18 strains. In addition to Chl a and the basic pigment load, all haptophytes analyzed contained either HFx (45 strains) or Chl c_1 (19 strains), but rarely both (only observed for six *Coccolithus pelagicus* strains). Classifications of calcifying and non-calcifying haptophyte species based on the HFx/Chl c_1 pigment pair coincided with taxonomy at the family level (Fig. 1) and with phylogeny (Fig. 2). Synthesis of HFx or Chl c_1 was thus identified as a primary differentiation of haptophyte pigment composition. DV-Chl c_3 , detected in 58 strains, was identified as an obligate companion of HFx, but was also found in 4 species (4 strains) containing Chl c_1 only. MV-Chl c_3 (49 strains) was never observed without its DV counterpart and frequently represented trace levels only, without a clearly visible peak in chromatograms obtained. The carotenoid 4-keto-HFx (39 strains) and an unknown Fx-like compound (FxL; 48 strains) were never detected without HFx. Mentioned relations did not, however, apply *vice versa*. Spectral characteristics and retention times of the carotenoids BFX and 4-keto-Fx were practically identical with the setup employed and a positive discrimination was not possible with the available results. Distribution of these compounds (Fig. 1) was, therefore, indicated as '4kFx/BFx'. Even so, distribution of the 4kFx/BFx compound (23 strains) was clearly restricted to families representing the order Coccochaerales. The non-polar Chl c_2 -MGDG_{E_{hux}} pigment was found in many cultures (59 strains). Chl c_2 -MGDG_{C_{poly}} appeared far less common (20 strains) and was usually detected in association with Chl c_2 -MGDG_{E_{hux}} (with the exception of 2 strains). Overall results revealed an extraordinary diversity of carotenoids and chlorophyll types, greater than that observed for any other algal group. The present study did not reveal a trace of MV or DV-Chl c_{PAV} in any species analyzed, which supports the taxonomic separation of the Prymnesiophyceae and Pavlovophyceae classes. Details on pigment distribution in relation to current taxonomy are discussed below.

Pleurochrysidaceae and Hymenomonadaceae

In addition to Chl a , most members of the family Pleurochrysidaceae Fresnel et Billard, classified in the order Coccochaerales Hackel emend. Young and Bown, synthesized Fx, Ddx, Dtx, β , β -carotene, MgDVP, Chl c_1 and Chl c_2 only. These compounds comprised the simplest pigment composition observed in the present study. Exactly the same composition has been described for *Pavlova lutheri* (Droop) Green, *Pavlova virescens* Billard and *Diacronema vlkianum* Prauser

emend. Green et Hibberd, classified in the class Pavlovophyceae (Van Lenning et al. 2003). According to the four different haptophyte pigment types defined by Jeffrey and Wright (1994) and the more detailed seven and nine type schemes defined by Garrido (1997) and Rodríguez (2001), these species would group together in "haptophyte type 1".

Coccolith structure and cytology indicate certain similarities between the families Hymenomonadaceae Senn and Pleurochrysidaceae, and indeed these species were initially classified together in one family (Fresnel and Billard 1991). These

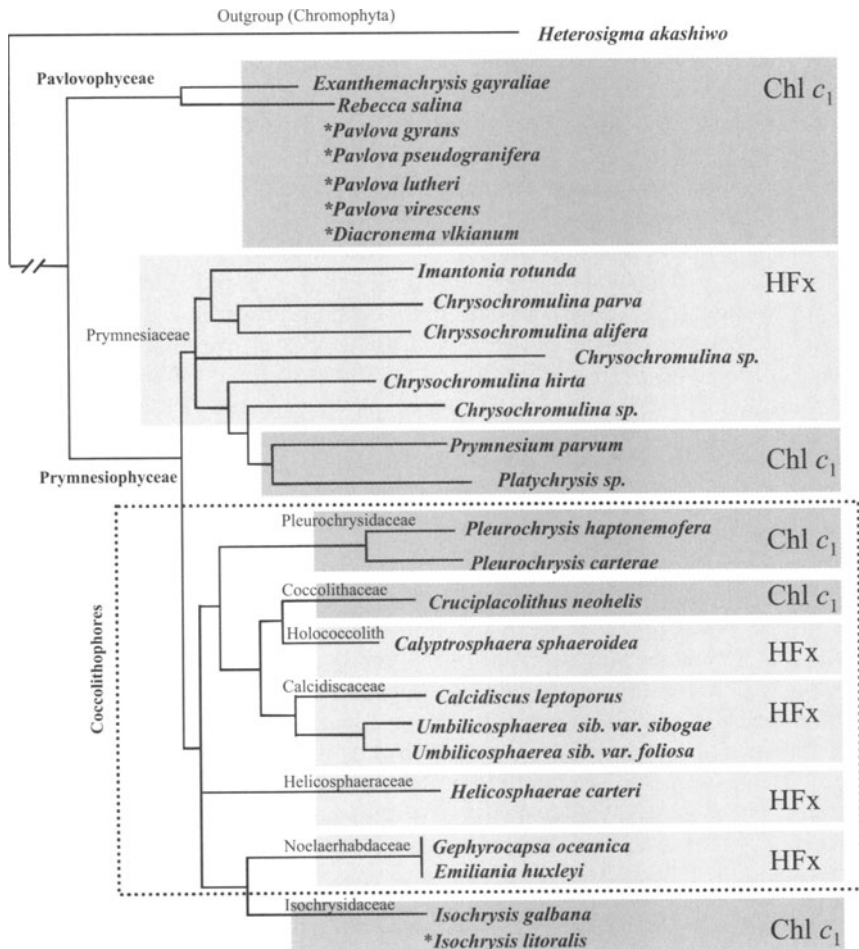


Fig. 2. Distribution of HFX and Chl c_1 among the Haptophyta in relation to published molecular phylogeny (Fujiwara et al. 2001). Species marked with * were not submitted to genetic studies. Pigment data on the genera *Imantonia*, *Chrysochromulina*, *Prymnesium* and *Platyachrysis* were derived from species available in the ALGOBANK, Caen, France.

similarities were perfectly supported by the relatively simple pigment composition of both families and the characteristic absence of an *E. hux*- or *C. pol*-type non-polar Chl c_2 (except *Ochrosphaera verrucosa*).

Identical pigment signatures observed for species representing the phylogenetically distant Pleurochrysidaceae and Pavlovophyceae (Fig. 2) are remarkable since the latter clearly diverged from the Prymnesiophyceae prior to the evolution of the coccolithophores (dated at >200 Ma using a molecular clock approach; Medlin et al. 1997). Separation of the Pleurochrysidaceae and Hymenomonadaceae was dated at >25 Ma, using molecular clock age estimates (Sáez et al. 2003). If pigments shared by all members of the families Hymenomonadaceae and Pleurochrysidaceae and the class Pavlovophyceae were derived from a common evolutionary ancestor they should comprise the oldest and most conservative components of the (haptophyte) light-harvesting system. However, an independent evolution of these pigments cannot, of course, be excluded.

Pleurochrysis carterae var. *dentata* Johansen et Doucette and *Pleurochrysis gayraliae* (Beuffe) Jordan et al. differ from the other members of the genus *Pleurochrysis* in containing trace amounts of 4kFx/BFx, and are in this respect similar to members of the Hymenomonadaceae. This variety was recently elevated to species level by Sáez et al. (2003), and the slight difference in pigment composition supports this conclusion. The presence of 4kFx/BFx in all members of the Hymenomonadaceae analyzed here also supports the separation, initially based on relatively minor differences in coccolith structure (Fresnel and Billard 1991), of these two families (with the exception of *P. carterae* var. *dentata* and *P. gayraliae*).

Taxonomic classification of *Jomonolithus littoralis* Inouye et Chihara, currently a genus and species *Incertae sedis*, in either the Pleurochrysidaceae or Hymenomonadaceae is still under discussion (Inouye and Chihara 1983; Billard and Inouye this volume). Observed 'type 1' pigment composition of *J. littoralis* suggested the Pleurochrysidaceae as the preferred location for this species and it was, therefore, classified with members of this family (Fig. 1).

The pigment content of *Ochrosphaera verrucosa* Schussnig and *Ochrosphaera neapolitana* Schussnig, classified in the Hymenomonadaceae, closely resembles that of the genus *Hymenomonas*, but 4kFx/BFx is no longer a trace compound, whereas DV-Chl c_3 is found in addition. This polar compound is not detected in other members of the Hymenomonadaceae or Pleurochrysidaceae, but is common for all other coccolith-bearing haptophytes analyzed. *Ochrosphaera verrucosa* is the only member of these two families containing detectable amounts of Chl c_2 -MGDG_{EHux}.

Coccolithaceae

The family Coccolithaceae Poche is represented by *Crucioplacolithus neohelis* (McIntyre et Bé) Reinhardt and two *Coccolithus pelagicus* morphotypes, previously differentiated on the basis of cell- and coccolith sizes (Baumann et al. 2000;

Geisen et al. 2002). Molecular clock calculations (Sáez et al. 2003) dated the divergence of *Cruciplacolithus* and *Coccolithus* at >25 Ma, whereas differentiation of the *C. pelagicus* morphotypes was estimated at ≈2 Ma only. The observed differences in pigment composition supported these conclusions.

The pigment content of *Cruciplacolithus neohelis* more closely resemble that of *Ochrosphaera* than *Coccolithus pelagicus*, although it coincides with the latter in synthesizing β,ε-carotene and Chl c_2 -MGDG_{Cpoly}. The five large-sized *Coccolithus pelagicus* strains synthesize all pigments detected in *Cruciplacolithus neohelis*, and contained MV-Chl c_3 , HFX, 4-keto-HFX and Chl c_2 -MGDG_{Ehux} in addition. Detectable amounts of FxL were only observed in two large-sized strains.

The pigment content of the large-sized *Coccolithus pelagicus* morphotype represents the most complex composition observed, and their simultaneous synthesis of HFX and Chl c_1 is unique. This composition has not previously been reported for any haptophyte species studied. The minor differences between large-sized strains are restricted to distribution of FxL (trace levels).

Analyses of the non-calcifying, flagellated stage (not previously considered in the literature for any coccolithophore) of strain KL2 yielded exactly the results as observed for its coccolith-bearing stage. The pigment composition of *Coccolithus pelagicus* is thus not affected by changes from diploid to haploid life cycle cell stages. This is also the case for *Emiliania huxleyi* discussed below.

The small-sized *Coccolithus pelagicus* strain contains HFX without a trace of Chl c_1 and neither MV-Chl c_3 nor β,ε-carotene are observed. This less complex pigment composition closely resembles those of the group of holococcolithophores analyzed here and members of the family Calcidiscaceae Young et Bown (discussed below).

Variations in pigment content observed between the genera *Cruciplacolithus* and *Coccolithus* exceed those detected within the other families studied. These results put into question their current taxonomic classification in a single family. Based on pigment signatures the Chl c_1 -containing *Cruciplacolithus* is separated from the HFX-synthesizing Coccolithaceae (Fig. 1). Although the suggested split is open for discussion, molecular studies for the other haptophyte groups (Fig. 2) perfectly support the taxonomic separation based on the Chl c_1 /HFX pair.

Holococcolithophores

The holococcolithophores do not comprise a family of separate species, but are currently considered to be haploid stages of the heteromorphic haplo-diplontic life-cycle common in haptophytes (Billard and Inouye this volume). Since it remains unknown to which family the holococcolithophores analyzed here actually belong, they are placed as a 'floating-group' (indicated with a dotted line; Fig. 1) in the order Coccochaerales to which they show closest genetic similarities (Fig. 2). The holococcolithophores analyzed do not contain a trace of β,ε-carotene (as is the case for the small-sized *Coccolithus pelagicus* morphotype), but contain high levels of MV-Chl c_3 (similar to members of the genus *Umbilicosphaera* Lohmann, classified in the Calcidiscaceae, see below). However, all holococcolithophores

analyzed contained 4-keto-HFx, which is common for all members of the Coccolithaceae, but absent in *Umbilicosphaera* and other member of the Calcidiscaceae. The unknown carotenoid FxL was detected in all holococcolithophores. Pigment signatures thus support the taxonomic location of the holococcolithophores analyzed between the Coccolithaceae and the Calcidiscaceae (Fig. 1).

Calcidiscaceae

The pigment content of this family differs from other Cocco-sphaerales by low- (*Umbilicosphaera* and *Oolithotus* Reinhardt) or undetectable amounts (*Calcidiscus leptoporus* (Murray et Blackman) Loeblich Jr et Tappan) of Fx. This was also the case for the four families classified in the orders Zygodiscales Young et Bown and Syracosphaerales Hay (discussed below), but Fx comprised one of the major compounds in all other coccolithophores analyzed. These results indicate that, in this case, low concentrations of Fx most likely have a genetic background.

Separation of the Calcidiscaceae into the three genera considered here coincides with the distribution of MV-Chl c_3 . This pigment is abundant in *Umbilicosphaera*, a trace compound in *Oolithotus*, and completely absent in *Calcidiscus*. This classification also correlates with the distribution of Fx (found in *Umbilicosphaera* and *Oolithotus*) and 4kFx/BFx (detected in *Oolithotus* and *Calcidiscus*).

Zygodiscales and Syracosphaerales

The pigments 4kFx/BFx and Chl c_2 -MGDG_{Cpoly} have not been detected in species comprising the families Zygodiscales and Syracosphaerales and Fx remained low or undetectable. Observed variation in pigment signatures is restricted to β , ϵ -carotene (absent in *Scyphosphaera apsteinii* Lohmann and *Algirosphaera robusta* (Lohmann) Norris), MV-Chl c_3 (not detected in *Helicosphaera carteri* var. *hyalina* (Gaarder) Jordan et Young) and 4-keto-HFx (only found in *H. carteri* var. *hyalina* and *Coronosphaera mediterranea* (Lohmann) Gaarder). Based on the Fx content (resembling that of *Umbilicosphaera*, *Oolithotus* and *Calcidiscus*) and detection of 4-keto-HFx (common for *Emiliania huxleyi*) in two strains the orders Zygodiscales and Syracosphaerales are placed (Fig. 1) between the Calcidiscaceae and Noelaerhabdaceae (discussed below).

Noelaerhabdaceae and Isochrysidaceae

Pigment signatures of the Noelaerhabdaceae are similar to those of other HFx-containing coccolithophores, but FxL is far more abundant. The 13 *E. huxleyi* strains from the CCMP culture collection practically have lost the ability to synthesize coccoliths, but they contain exactly the same pigments as observed in the

coccolith-bearing strains (isolates more recently). Detectable differences associated with depth of isolation, ranging from surface (CCMP 377 and 378) to 1000 meter (CCMP375), are not observed either. Differences between *Emiliania huxleyi*, *Emiliania* type R and *Gephyrocapsa oceanica* Kamptner are of minor importance. Considering the close genetic distances between *Emiliania* and *Gephyrocapsa oceanica* Kamptner (Fig. 2) this result was expected. The pigment β, ϵ -carotene, common for all *Emiliania* strains (and type R strain TQ26), but undetectable in *Gephyrocapsa oceanica*, represents the only difference. Analyses of haploid and diploid phases of the TQ26 strain reveal exactly the same pigments, providing further evidence for a genetically, rather than environmentally, controlled pigment load.

The classification of the Isochrysidaceae (the only non-coccolith bearing haptophytes employed) and the Noelaerhabdaceae families in the same order Isochrysidales (Fig. 1) is supported by phylogenetic results (Fig. 2). However, the pigment composition of the Chl c_1 -synthesising Isochrysidaceae is quite different from the HFx-containing Noelaerhabdaceae, and more closely resembles that of *Pleurochrysis* (except for Chl c_2 -MGDG_{E_{hux}}). As shown below, these discrepancies likely reflect an evolutionary differentiation of coastal and open-water haptophytes.

HFx, Chl c_1 and ecological preferences

Distribution of Chl c_1 and HFx coincides almost perfectly with current taxonomy at the family level (Fig. 1) and with phylogeny (Fig. 2). Classification of the Chl c_1 -synthesising *Cruciplacolithus neohelis* and the HFx-containing small-sized *Coccolithus pelagicus* strain in the same family is the only exception.

A remarkable coincidence is that *Cruciplacolithus neohelis* and all other species typically found in nearshore locations (coastal, benthic or brackish) contain Chl c_1 , rather than HFx. This general rule applies to both calcifying- (*Pleurochrysis*, *Cruciplacolithus*, *Jomonolithus*, *Hymenomonas* and *Ochrosphaera*) and non-calcifying genera (*Prymnesium*, *Platychrysis* and *Isochrysis*), and even holds true for members of the class Pavlovophyceae.

Members of the HFx-synthesizing genera (such as *Coccolithus pelagicus*, *Emiliania huxleyi* and *Gephyrocapsa oceanica*), on the other hand, always are characteristic of open-oceanic regions, although their biogeographical distribution may well include open-coastal (i.e. not littoral) locations.

Content of Chl c_1 as a possible indicator for a nearshore origin of a haptophyte has not previously been reported and the functional advantage of this compound for such locations remains unclear. However, this phenomenon deserves further investigation as it perfectly supports field distribution of pigments observed along sections perpendicular to the African coast, south of the Canary Islands archipelago (Van Lenning 2000).

Conclusions

The extraordinary diversity in pigment content observed for the coccolithophores can be ordered according to large-scale phylogenetic groupings and/or general ecological preferences, while similarities clearly increase towards the lower taxonomic levels. Pigment compositions of coccolithophores are not affected by changes from diploid to haploid life cycle cell stages, and different strains from the same species have always yielded identical results. Overall results show that variations in haptophyte pigment composition have an evolutionary origin, rather than reflecting short-term temporal changes in environmental conditions. Evolutionary modifications of the light harvesting system may, on the other hand, very well result from adaptations to ecological conditions.

Based on the characteristic association between synthesis of Chl c_1 and ecological preferences, HFx recovered its previously questioned status as a straightforward marker for haptophyte distribution in open coastal and oceanic habitats. However, this approach is only valid when Chl c_1 is not detected. This is a common situation for offshore locations, but should be confirmed in samples from other areas. This fact recalls, once more, the need for implementation of adequate chromatographic procedures, capable of separating Chl c_1 from Chl c_2 and MgDVP. When concentrations of Chl c_1 reach detectable levels (expected to occur in coastal areas) a more complicated approach, considering all pigments present, is required. This task is now commonly performed with the CHEMTAX program (Mackey et al. 1996), which interprets field data by means of user provided pigment data observed in cultured representatives.

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***Emiliana huxleyi*: bloom observations and the conditions that induce them**

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Summary

Most of what is known about the distribution of blooms of *Emiliana huxleyi* comes from satellite evidence. However, patches of bright water in satellite images are not always *E. huxleyi* blooms and satellite evidence needs to be verified by in situ sampling in the area. In this article we firstly describe the observational evidence for these blooms in various regions of the global ocean, and then proceed to describe mimicking conditions: the occasional bright waters that are not *E. huxleyi* blooms. In the second part of this article we discuss the possible causes of the *E. huxleyi* blooms. We review the various hypotheses concerning the water conditions required to generate these blooms.

Introduction: distribution and environmental effects

Emiliana huxleyi (Fig. 1) is an extremely cosmopolitan coccolithophore species. In addition to the spectacular blooms that are enumerated below, it occurs in lesser (but still significant) numbers in all oceans except the Arctic Ocean and high-latitude Southern Ocean (Winter et al. 1994), with either complete absence or just a few (remnant?) cells observed in the latter two areas (e.g. Winter et al. 1999; Findlay and Giraudeau, 2000). While *E. huxleyi* blooms occur in relatively eutrophic regions (e.g. following diatom spring blooms in temperate latitudes), the species is also rather numerous in the permanently oligotrophic waters of the subtropical gyres. It is frequently the most numerous species in phytoplankton cell counts from surface water samples, although because of the rather small size of the cells (circa 5 μm in diameter), it makes a lesser contribution to total biomass. In fact, for the same reason, *E. huxleyi* blooms are usually associated with low rather than high chlorophyll-*a* concentrations. ‘Bloom’, as generally used, is a

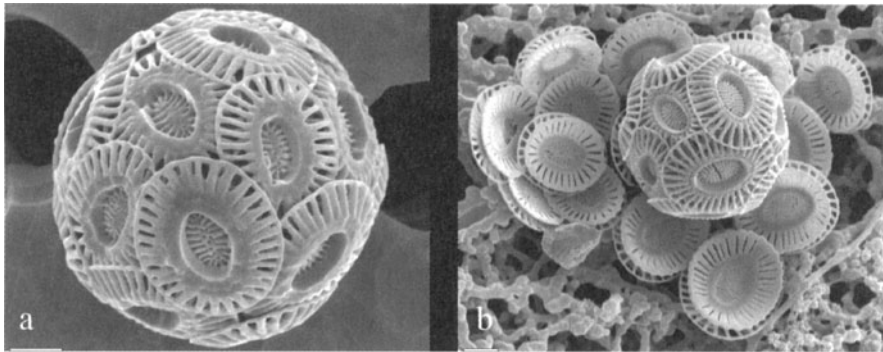


Fig. 1. *Emiliana huxleyi* cell **a.** surrounded by attached coccoliths, and **b.** with coccoliths shedding off (images courtesy of Markus Geisen and Jeremy Young).

rather imprecise term, but in this paper it is defined, arbitrarily, to refer only to *E. huxleyi* cell concentrations of at least 1,000,000 per liter. Blooms can cover very large areas, for instance at least 200,000 km² in the Eastern Bering Sea and Bering Straits in 1997 (Sukhanova and Flint 1998), and 250,000 km² in the North Atlantic south of Iceland in 1991 (Holligan et al. 1993a). Cell concentrations vary between blooms and according to the stage of the bloom (sometimes bright waters contain

Table 1. Field evidence verifying that many areas of bright waters in satellite images correspond to areas where very high *Emiliana huxleyi* cell numbers are observed.

Bloom Location	Concentration (10 ⁶ cells litre ⁻¹)	Year	Source
Norwegian fjords	10 – 100	1950s	Birkenes and Braarud 1952
	up to 115	1955	Berge 1962
	up to 7	1992	Kristiansen et al. 1994
North Sea	<0.1 – >1	'80s	Holligan et al. 1993b
	0.1 – 1.2	1993	Van der Wal et al. 1995
	1 – 6	1994	Head et al. 1998
	0.6 – 2.3	1999	Burkill et al. 2002
Western English Channel	up to 8.5	'80s	Holligan et al. 1983
	up to 2	1992	Garcia Soto et al. 1995
Bay of Biscay	up to 3	1998	Lampert et al. 2002
North Atlantic	up to 4	1987	Malin et al. 1993
	0 – 10	1991	Holligan et al. 1993a
Gulf of Maine	<0.5 – >2	1988–89	Balch et al. 1991
	>1	1988–90	Townsend et al. 1994
Nova Scotian shelf	Up to 1.5	1991	Brown and Yoder 1993
Black Sea	Up to 10	1992	Mankovsky et al. 1996 (cited in Cokacar et al. 2001)
	4.7, 31	1990, 93	Mihnea 1997
Bering Sea	2.1 – 2.8	1997	Sukhanova and Flint 1998

abundant light-scattering coccoliths left over from a cell population that has mostly died). *E. huxleyi* is unusual amongst coccolithophores in that under certain environmental conditions the cells *overproduce* coccoliths (Paasche 2002), leading to shedding of excess coccoliths and very large concentrations of detached coccoliths in the water. Light scattering is by both detached coccoliths and coccoliths within coccospheres. Some example measured cell concentrations are given in Table 1. The highest concentration ever reported is 115,000,000 cells per liter, from a Norwegian fjord in 1955 (Berge 1962) (Table 1).

The blooms have significant regional environmental impacts (Westbroek et al. 1993), via increased water albedo (reflectance) (Tyrrell et al. 1999), DMS production (Malin and Steinke this volume), large fluxes of calcium carbonate out of the surface waters and changes in the oceanic uptake of CO₂ (Rost and Riebesell this volume). Because of the light scattering properties of the coccoliths, bloom water is very turbid. This leads to increased light/heat trapping in the surface layers, decreased light/heat penetration to depth, and increased reflection of light/heat back out of the sea surface (Tyrrell et al. 1999). The impacts of the blooms on ocean-atmosphere exchange of CO₂ has been investigated on several occasions (Robertson et al. 1994; Van der Wal et al. 1995; Murata and Takizawa 2002).

History of bloom observations

To the best of our knowledge, the first reported observations of *E. huxleyi* blooms and associated milky-turquoise waters came from fjords on the western coast of Norway (Birkenes and Braarud 1952; Berge 1962). Blooms continue to occur in Norwegian waters at the present time, including offshore out to many miles, as shown for instance in a satellite image from May 2000 in which an enormous bloom stretches up from the Skaggeak and a long way up the western Norwegian coastline <http://www.soes.soton.ac.uk/staff/tt/eh/pics/sat/skag2.jpg>.

As satellites started being used to look at the sea, *E. huxleyi* blooms started being picked up in these images. The possibility that large areas of the open ocean could be significantly paler in color was simply not appreciated to any great extent (although see Hardy 1956) before these satellite images were available. Some of the first satellite studies concentrated on areas to the west of the English Channel (Holligan et al. 1983), or off the west coast of France http://www.soes.soton.ac.uk/staff/tt/eh/v_0.htm. High-resolution modern SeaWiFS (Table 2) images show that small *E. huxleyi* blooms occur all along the NW European shelf break from the Bay of Biscay up to the west coast of Ireland <http://www.soes.soton.ac.uk/staff/tt/eh/pics/sat/three.tif>. Whereas probably the first *E. huxleyi* bloom ever to be seen in a satellite image was in a LANDSAT-1 (Table 2) image http://www.soes.soton.ac.uk/staff/tt/eh/v_1.htm, later studies made use of AVHRR (Table 2) images, for on the Nova Scotian shelf and Grand Bank (Brown and Yoder 1993), in the Gulf of Maine (Balch et al. 1991) and in the North Sea (Holligan et al. 1993b). Much of the first biological and optical information was obtained in the Gulf of Maine (Balch et al. 1991). In 1991 an enormous bloom was seen in

AVHRR images south of Iceland, covering 250,000 km² (Holligan et al. 1993a), and ships traveled from the UK to sample it (Holligan et al. 1993a) and to measure its impact on carbon dioxide concentrations (Robertson et al. 1994). Blooms are still seen south of Iceland in SeaWiFS images <http://www.soes.soton.ac.uk/staff/tt/eh/pics/sat/natl1.jpg>

Other early satellite work with *E. huxleyi* blooms involved the development of an automatic technique to scan CZCS images for the presence of coccolithophore blooms (Brown and Yoder 1994). CZCS images were rather low resolution (each individual pixel corresponded to a rather large area of the Earth's surface) but CZCS's global coverage allowed construction of a global map of where *E. huxleyi* blooms (or at least white waters, most of which are *E. huxleyi* blooms) occur (Brown and Yoder 1994). The global map from the CZCS years (1978–86) gave us our first wide-ranging insight into the distribution of blooms of any single phytoplankton species. As expected from previous work, the North Sea and northern North Atlantic areas showed up as areas of particularly high *E. huxleyi* activity. The North Pacific was shown to be generally less favorable for *E. huxleyi* blooms than the North Atlantic. Less expected was the discovery that *E. huxleyi*

Table 2. Satellites used for *Emiliania huxleyi* bloom observations.

Acronym	Full name	Dates of operation	Number of visible wavebands	Spatial resolution (km)
LANDSAT*	LAND remote-sensing SATellite	1972 – still operating	3	0.03
CZCS	Coastal Zone Color Scanner	1978 – 1986	4	18 [†]
AVHRR	Advanced Very High Resolution Radiometer	1978 – still operating	1	4 [†]
OCTS	Ocean Color and Temperature Scanner	08/1996 – 07/1997	6	0.7
SeaWiFS	Sea viewing Wide Field of view Sensor	1997 – still operating	6	4.5 or 9 [†]
MODIS	MODerate resolution Imaging Spectroradiometer	1999 – still operating	10	1 or 5 [†]
MERIS	MEDium Resolution Imaging Spectrometer instrument	2002 – still operating	8	1 or 5 [†]

* there have been several generations of LANDSAT sensors. LANDSAT-1 started its operation in 1972 and ended in 1978. The last one, LANDSAT-7, was launched in 1999.

[†] the resolution of images frequently examined for the presence of coccolithophore blooms, rather than the maximum resolution of the instrument (e.g. see Fig. 3, Brown and Yoder 1994).

blooms apparently blanket the Patagonian shelf (between Argentina and the Falkland Islands) on a regular basis, as also seen in recent SeaWiFS images during November and December of different years <http://www.soes.soton.ac.uk/staff/tt/eh/pat.html>. Elsewhere in the southern hemisphere *E. huxleyi* blooms were detected less frequently than in the northern hemisphere (Brown and Yoder 1994). Brown and Yoder recognized from the start the possibility of certain water conditions masquerading as *E. huxleyi* blooms (see below for more discussion), and therefore that their global map was not foolproof.

Since 1997 the "tool of choice" for sensing *E. huxleyi* blooms has been the SeaWiFS sensor (Table 2), although new sensors such as MODIS and MERIS (Table 2) are proving equally capable of detecting the blooms. As well as confirming blooms in locations where CZCS saw them, SeaWiFS has also returned images of what appear to be extensive *E. huxleyi* blooms where CZCS saw none. In the Barents Sea, for example, where CZCS detected no blooms (although this area is near the sensor's latitudinal limit) SeaWiFS has seen many extensive areas of pale waters (July–September 1997–2002) that are most likely *E. huxleyi* blooms <http://www.soes.soton.ac.uk/staff/tt/eh/bar.html>. There was apparently little *E. huxleyi* activity in the Black Sea during 1978–86, according to CZCS (Brown and Yoder 1994), but there have since been many basin-wide blooms (Cokacar et al. 2001). SeaWiFS has also seen many areas of moderately pale waters north of the Antarctic Polar Front in the Southern Ocean, for instance between Tasmania and New Zealand <http://www.soes.soton.ac.uk/staff/tt/eh/pics/sat/tasnz.jpg>. The biggest surprise of the last few years, however, has been the dramatic appearance of *E. huxleyi* in the eastern Bering Sea <http://www.soes.soton.ac.uk/staff/tt/eh/ebs.html>. In some of the very first images that SeaWiFS sent back to Earth in September 1997, virtually the whole of the eastern Bering Sea (over the continental shelf) was blanketed by a dense *E. huxleyi* bloom that persisted for many months, approximately from July to October. Local fishermen asserted that the phenomenon was completely new, which has been largely substantiated by a detailed analysis of earlier satellite images of the region (Merico et al. 2003). Careful inspection of CZCS images in 1978–86 and AVHRR images in 1987–1996 revealed no evidence of earlier *E. huxleyi* blooms. Nonetheless, a small patch of bright water (almost certainly, from its location and persistence, a patch of *E. huxleyi*) was visible to the south of St. Matthew Island from the 26th of August to the 3rd of October 1996 (Merico et al. 2003). This small bloom was probably a precursor to the much larger blooms starting the following year.

Intense and widespread *E. huxleyi* blooms also occurred in the eastern Bering Sea in 1998, 1999 and 2000, although not in 2001 or 2002. It is not clear as yet what impact these blooms have on the higher trophic levels of the Bering Sea ecosystem. However, some effects have been observed and considered as direct consequences of these events. In particular, a massive die-off of short-tailed shearwaters took place in summer 1997 (Baudini et al. 2001). The lower body mass and lipid values of the birds suggested starvation as the most likely cause of death. The fact that most of the carcasses were found within the extent of the coccolithophores bloom (Baudini et al. 2001) therefore indicated that the chalky

(very turbid) waters might have played a role in reducing the ability of the birds to spot their prey from above the sea-surface.

A comprehensive and up-to-date coverage of where *E. huxleyi* blooms are occurring at the moment, and have occurred since 1997, can be obtained at Chris Brown's website http://orbit-net.nesdis.noaa.gov/orad2/doc/ehux_www.html.

All that glitters is not *E. huxleyi*

Not all bright waters are caused by *E. huxleyi* blooms. Alternative causes of bright waters are discussed in this section. What has been perhaps even more surprising about SeaWiFS images of the eastern Bering Sea is that blooms appear to have been taking place in the middle of winter (Iida et al. 2002). Given the high latitude, approximately between 56°N and 60°N, wintertime blooms of any phytoplankton should be impossible. According to a well-substantiated theoretical understanding of phytoplankton dynamics in temperate locations experiencing spring blooms, of which the eastern Bering Sea is one, deep mixing and low surface sunlight levels should make net growth of phytoplankton (photosynthesis exceeding respiration) impossible in wintertime (e.g. Sverdrup 1953). But SeaWiFS and OCTS images show bright water patches resembling *E. huxleyi* blooms during for instance February, March, April and May from 1998 to 2000 (Iida et al. 2002; Fig. 1, Broerse et al. 2003), in all cases at times before the spring blooms took place in those years. Because of surprise at these apparent *E. huxleyi* blooms in winter, sampling was undertaken in February 2001 in order to be able to observe what was in the water and imparting the pale turquoise color (Broerse et al. 2003). The results were surprising.

Instead of seeing multitudes of coccoliths, we found instead that, at the brightest station on the transect, coccoliths were strongly outnumbered by empty (no living cells inside) diatom frustules (Fig. 2). From several lines of evidence it was determined that the diatom frustules were remnants from earlier blooms in the year, that had settled to seafloor but had then been resuspended by strong winds during stormy weather. The seafloor is not very deep over the continental shelf (average about 70 meters). Many of the frustules were broken up so that we were seeing more fragments than whole frustules. On the basis of measurements of the scattering properties of opal material, it was calculated that the observed concentration of diatom frustules and fragments was sufficient to produce the sea-surface brightness seen in the satellite images (Broerse et al. 2003).

These observations suggest that resuspension of broken-up diatom tests may also, perhaps, be responsible for other anomalous bright water patches, for instance in winter over the Grand Banks on 03 Mar 2001 <http://visibleearth.nasa.gov/cgi-bin/viewrecord?7769> and 28 Jan 2002 <http://visibleearth.nasa.gov/cgi-bin/viewrecord?11785>. Other coccolithophore species can also turn the waters pale if blooming in sufficient numbers. For instance, the best documented example is of a bloom of *Gephyrocapsa oceanica* (Blackburn and Cresswell 1993) in the

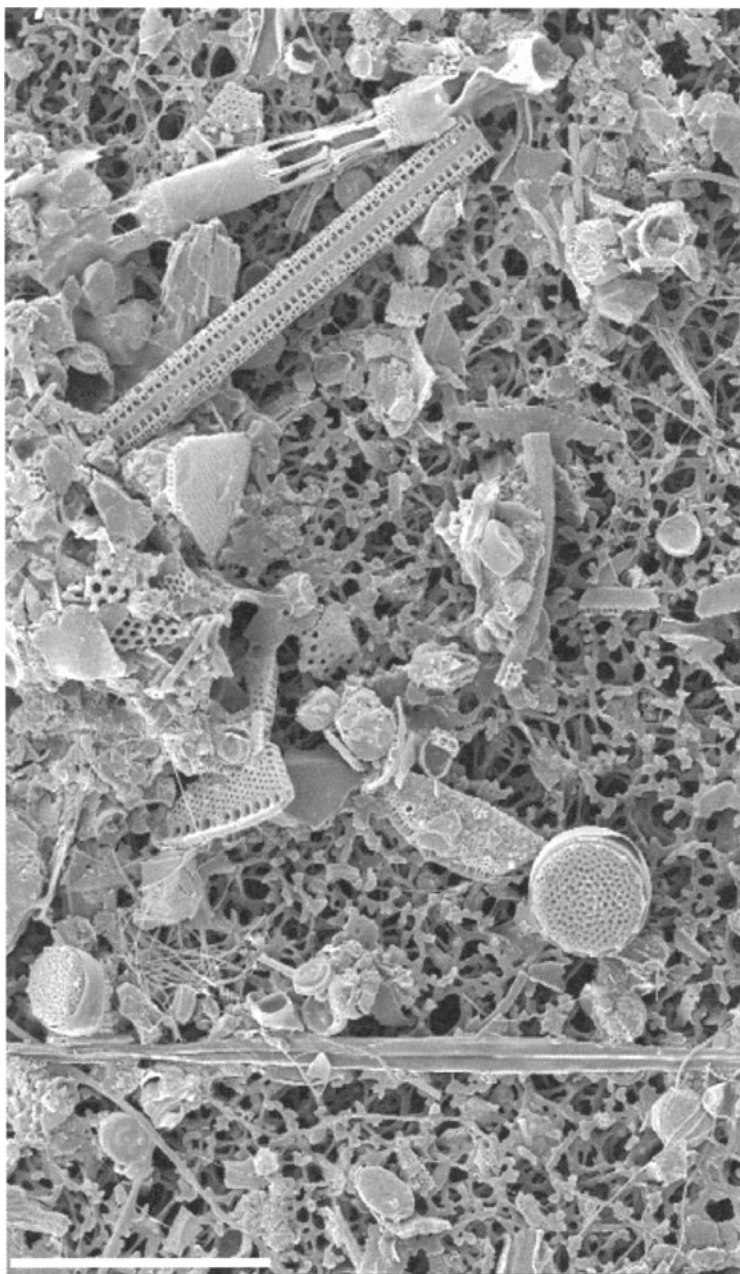


Fig. 2. Scanning electron microscope image from a wintertime bright water station in the eastern Bering Sea (Broerse et al. 2003). The suspended matter is dominated by broken up diatom frustules.

coastal embayment of Jervis Bay on the east coast of Australia in mid December 1992, of which a striking photograph was taken (Blackburn and Cresswell 1993). In general, however, *E. huxleyi* is thought to be unique in overproducing coccoliths and then shedding the excess ones into the water (Paasche 2002), so it is likely that hardly any of the open-ocean bright waters are attributable to species other than *E. huxleyi*.

Other water conditions known to be able to mimic *E. huxleyi* blooms in appearance are: (1) shallow carbonate shelves such as in the Gulf of Carpentaria (Brown and Yoder 1994), and the Grand Banks of the Bahamas, (2) suspended sulphur granules (Weeks et al. 2002), and (3) glacial rock flour in some high-altitude lakes. Suspended sediment, for instance at river mouths, does not as a rule look the same as *E. huxleyi* blooms, because there is in this case usually a brownish tinge to the water color. Live diatom blooms (when the cells still occupy the frustules, as opposed to the wintertime Bering Sea case above) are usually much darker and browner (due to pigments in the diatoms) than are *E. huxleyi* blooms.

Because of the possibility of mimicking conditions, it is important to obtain in situ verification of the cause of each location of bright waters at each time of year. So, for instance, the summer/autumn bright waters have been positively identified (see Table 1 for more information) as *E. huxleyi* blooms in the Norwegian fjords (e.g. Berge 1962), in the North Sea (Holligan et al. 1993b; Van der Wal et al. 1995), in the North Atlantic (Holligan et al. 1993a), in the Bering Sea (Sukhanova and Flint 1998), in the Gulf of Maine (Balch et al. 1991), and in the Black Sea (Mihnea 1997; Mankovsky et al. 1996).

A major area of white water that appear to be an *E. huxleyi* bloom but for which in situ confirmation is still urgently needed is the Patagonian Shelf.

What causes *Emiliana huxleyi* blooms?

The second part of this article will concern the question as to which water conditions are conducive to the development of *Emiliana huxleyi* blooms. To put it another way, what is the ecological niche for *E. huxleyi*? We will now review several hypotheses for the environmental conditions that favor *E. huxleyi* blooms, the reasons for those hypotheses and the evidence in support of them. The ecology of all coccolithophores is also discussed in other chapters (Balch this volume; Rost and Riebesell this volume). The physiology of *E. huxleyi* is discussed in detail elsewhere (Paasche 2002; Brownlee and Taylor this volume) and is not covered here.

High light

The first hypothesis is that high light conditions trigger *E. huxleyi* blooms. Evidence for this hypothesis was put forward by Nanninga and Tyrrell (1996), and is partially recapitulated here, together with more recent evidence. A literature search of reports of natural blooms of *E. huxleyi* (and the associated measured

water conditions) reveals that they all occur in highly stratified water where the mixed layer depth is usually ~10–20 m, and is always ≤ 30 m (Nanninga and Tyrrell 1996). As an example, the most intense bloom ever reported in the literature occurred in a fjord SE of Norway in May–July 1955 (Berge 1962). There were a staggering 115,000,000 *E. huxleyi* cells per liter. There was very little monitoring of the environment prior to the bloom, but Berge noted:

“... in the summer of 1955, the intensity and extent of the discoloration was so remarkable that quite unusual ecological conditions must have existed. ... The tentative conclusion is drawn that high light intensities during May and June 1955 acted selectively on the plankton, giving *E. huxleyi* advantageous conditions.”

Some of the best data we have on conditions leading to *E. huxleyi* success in competition with other phytoplankton comes from mesocosms in Norwegian fjords, run by Jorun Egge and others at the University of Bergen. Fig. 3 shows the sizes of *E. huxleyi* and *Phaeocystis* populations in mesocosm bags having experienced different nutrient and light conditions. The initial water in the bags was fjord water with an unaltered natural assemblage of phytoplankton. Although many phytoplankton species were present, only the final populations of *E. huxleyi* and *Phaeocystis* are shown for clarity. We can see that the large black circles (*E. huxleyi* blooms) in Fig. 3 occur mostly to the top of each plot, i.e. following higher-than-average light intensities.

The large *E. huxleyi* bloom south of Iceland in 1991 was modeled and high light was shown to be a possible cause (Tyrrell and Taylor 1996). Fig. 4 shows the temperature structure of the water along a north-south transect through the bloom. To the north of the transect where the bloom was most intense, so too is the stratification. Mixing is not very deep and so average light intensities in the surface layer are high.

The most notable difference to the environment of the eastern Bering Sea in 1997, the year of the first massive *E. huxleyi* blooms, was unusually strong stratification from June onwards, following a single strong storm in May (Stabeno et al. 2001). The bloom was first noted in early July, by scientists out at sea (Sukhanova and Flint 1998). A mooring station collected time series of temperature and salinity profiles data continuously through the beginning of the bloom as well as in previous and in subsequent years (Stabeno et al. 2001; Hunt and Stabeno 2002). The very strong stratification in June/July 1997 is apparent (Fig. 5, Hunt and Stabeno 2002).

Further objective evidence for the importance of stratification to the genesis of *E. huxleyi* blooms comes from recent work on the Black Sea. A study of satellite images (Cokacar et al. 2001) found that *E. huxleyi* blooms did not occur randomly with respect to the circulation structure of the Black Sea, but rather that *E. huxleyi* bright waters were more frequently associated with cyclonic as opposed to anti-cyclonic eddies. The surface waters of cyclonic eddies tend to be more strongly stratified than those of anti-cyclonic eddies.

An innovative recent study has compared *E. huxleyi* bloom occurrence on a global scale (as detected in SeaWiFS images using an automatic algorithm:

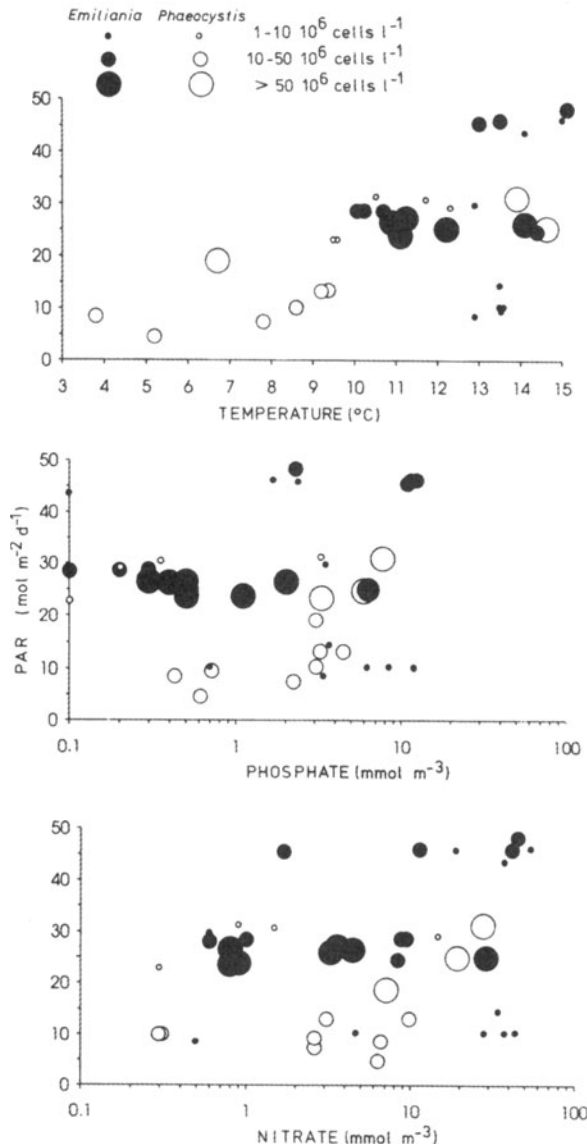


Fig. 3. Magnitudes of mesocosm blooms of *Emiliana huxleyi* and *Phaeocystis* versus several environmental variables (from Egge and Heimdal 1994). Light, nutrients and temperature are 5-day averages, over the five days prior to the measurement of phytoplankton concentration.

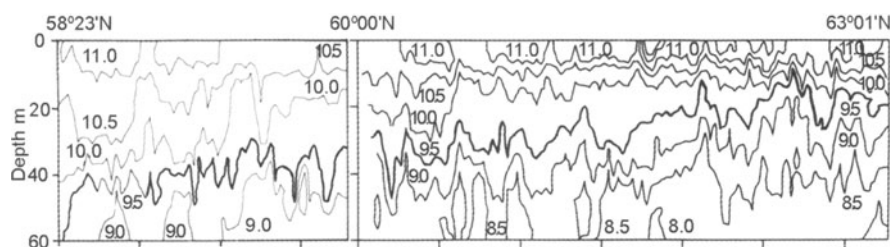


Fig. 4. Temperature along a north-south transect through an *Emiliania huxleyi* bloom south of Iceland, 1991 (taken from Holligan et al. 1993a). Highest *E. huxleyi* concentrations are towards the northern end of the transect.

http://orbit-net.nesdis.noaa.gov/orad2/doc/ehux_www.html, but with shallow low-latitude sites excluded because similar reflectance is generated by resuspended calcareous sediments) with datasets of physical and nutrient parameters (Iglesias-Rodriguez et al. 2002). The study involved an objective analysis of parameters correlated with *E. huxleyi* blooms, although such correlations do not necessarily imply causation (for instance some association between *E. huxleyi* blooms and stratified waters is to be expected due to an effect of the blooms on the water state rather than vice-versa). Their results suggest that *E. huxleyi* blooms are “confined primarily to nutrient-depleted, temperate, and high-latitude oceans with relatively high critical irradiances.” (Iglesias-Rodriguez et al. 2002).

Nanninga and Tyrrell (1996) suggested that the reason *E. huxleyi* outcompetes other phytoplankton at high light intensities is that *E. huxleyi* is uniquely tolerant of high light intensities. That is to say, *E. huxleyi* is not prone to photoinhibition, in contrast to other phytoplankton. Laboratory and field PI-curves in which *E. huxleyi* has been acclimatized to and tested at high light intensities show a lack of photo-inhibition, even at the highest light intensities likely to be encountered in nature (Balch et al. 1992; Nanninga and Tyrrell 1996). While unusual tolerance of high light intensities may turn out to be important (but see Stolte et al. 2000), the evidence is not conclusive and there is no accepted physiological reason (e.g. unique pigments) why *E. huxleyi* should outperform other phytoplankton at high light. It is known that coccoliths do not act as “sunscreens” (Paasche and Klave-ness 1970).

Low silicate

E. huxleyi is a fast-growing (*r*-selected) phytoplankton species, capable under favorable conditions of multiplying more rapidly than most other species. Most diatoms, however, can multiply even more rapidly (Furnas 1990). Diatoms are typically the fastest-growing of all phytoplankton and dominate in eutrophic (*r*’-selected) environments such as regions of upwelling, river mouths, spring blooms and oceanographic fronts as long as there is sufficient dissolved silicate. For non-diatom fast-growers like *E. huxleyi*, it seems plausible that they should do well in

eutrophic environments where diatoms are somehow excluded from the competition, for instance where nitrate, phosphate and other nutrients are still abundant but where dissolved silicate has been exhausted. In mesocosm experiments diatoms tend to dominate the phytoplankton community except when silicate is scarce (Egge and Aksnes 1992). Such a situation occurs towards the end of spring blooms in the northeast North Atlantic (Fasham et al. 2001, Fig. 12a), and *E. huxleyi* blooms also occur in this area (e.g. Holligan et al. 1993a). *E. huxleyi* blooms do not seem to occur in those parts of High Nutrient Low Chlorophyll regions (e.g. North Pacific, high-latitude Southern Ocean) where high concentrations of dissolved silicate persist year-round.

The probable importance of silicate (lack of it) to *E. huxleyi* is graphically illustrated by the case of the Black Sea. Comparison of the pattern of bright waters seen in 1978–86 by the CZCS satellite (Brown and Yoder 1994) with the pattern seen by the SeaWiFS satellite since 1997 (http://orbitnet.nesdis.noaa.gov/orad2/doc/ehux_www.html; Iglesias-Rodriguez et al. 2002) reveals that whereas CZCS detected only a few patches of bright water in the Black Sea, the whole of the Black Sea is bright in the SeaWiFS analysis. Although sediment records show that *E. huxleyi* has been present at some concentration in the Black Sea for about 1600 years (Hay et al. 1991), phytoplankton sampling records show a decline in diatom numbers and an increase in *E. huxleyi* numbers since the 1960s (Mihnea 1997; Humborg et al. 1997). What has caused the recent shift away from diatoms and towards *E. huxleyi* (and other flagellates)? Since 1970–72, when the Iron Gates Dam was constructed across the River Danube (which provides the majority of the freshwater inflow to the Black Sea), the river load of dissolved silicate into the Black Sea has markedly decreased while river load of nitrogen has increased. This has depressed dissolved silicate levels in the Black Sea, with wintertime concentrations decreasing from about 50 down to about 20 $\mu\text{Mol kg}^{-1}$ (Humborg et al. 1997). The large surplus of fixed nitrogen now left over following silicate depletion by spring diatom blooms is being removed by non-diatom species.

Phosphate more limiting than nitrate

Several studies have noted anomalous N:P ratios during *E. huxleyi* blooms. Typically, in the ocean as a whole, the N:P ratio in surface waters is generally rather low, certainly lower than the Redfield ratio of 16:1 (Fanning 1992). In the 1991 *E. huxleyi* bloom to the south of Iceland, surface layer N:P ratios were however similar to or greater than 16 in the bloom waters, and less than 16 further south (Tyrrell and Taylor 1996). In the Gulf of Maine in 1989 lowest phosphate levels were noted at bloom stations (Townsend et al. 1994) (ranging from 0.02 to 0.16 μM , vs. 0.21 to 0.49 μM at the non-bloom stations). Though Townsend et al. (1994) found that the phosphate concentrations were lower in bloom waters, they noted that N:P ratios were not different (ranging from 5.9 to 6.4 at bloom and non-bloom stations), although they were lower, in both bloom and non-bloom waters, than the Redfield ratio. Van der Wal et al. (1995) found in a bloom study in 1993 in the North Sea that phosphate concentrations at bloom stations were about 0.1

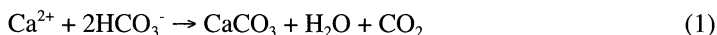
μM and about $0.3 \mu\text{M}$ at non-bloom stations. N:P ratios ranged from 6–11 in coccolithophore-rich waters and from 13–15 in non-bloom waters. Mesocosm experiments also observed *E. huxleyi* success (larger final populations) for those bags manipulated by adding lots of N and little P rather than vice-versa (Egge and Heimdal 1994), and it can be seen in Fig. 3 that most *E. huxleyi* blooms (large black circles) occurred at lower phosphate concentrations while the same cannot be said for lower nitrate concentrations. A modeling study of the mesocosms invoked *E. huxleyi* success at low phosphate concentrations to explain the observations (Aksnes et al. 1994).

From a physiological perspective *E. huxleyi* is known to be able to synthesize the enzyme alkaline phosphatase (Kuenzler and Perras 1965; Riegman et al. 2000), which allows uptake of some fractions of dissolved organic phosphates and should therefore impart an advantage to *E. huxleyi* when inorganic phosphate is limiting. “Competition experiments” have been carried out by growing inoculae of several phytoplankton strains in a single chemostat, and in several such experiments greater numbers of *E. huxleyi*, and greater percentages out of the total, were obtained at high N/P ratios rather than at low N/P ratios (Riegman et al. 1992).

However, recent examination of nutrient data from the eastern Bering Sea during the years of the *E. huxleyi* blooms there suggests that those blooms occurred when N was limiting but P was abundant, suggesting that low phosphate is not an absolute requirement for the occurrence of these blooms.

Low dissolved carbon dioxide

The chemical reaction for calcification is



As described by Brownlee and Taylor (this volume), the synthesis of calcium carbonate coccoliths therefore releases CO_2 as a by-product, and this extra CO_2 is probably available for photosynthesis. This indirect means of obtaining CO_2 from HCO_3^- could potentially give *E. huxleyi* an advantage over other phytoplankton if the rate of supply of external CO_2 is limiting for growth. Here this hypothesis is discussed from the ecological rather than the physiological point of view (see Rost and Riebesell this volume, for the latter). Although there is an abundance of dissolved inorganic carbon (DIC) in all ocean waters (usually more than $2000 \mu\text{Mol}$ compared to a maximum of about $30 \mu\text{Mol}$ of nitrate, so order 60 times more DIC than nitrate whereas phytoplankton only require about 7 C atoms for every N atom), at typical pHs most of this carbon exists as bicarbonate (HCO_3^- , about 90% of total DIC) and carbonate (CO_3^{2-} , about 10% of the total), with typically less than 1% as dissolved carbon dioxide ($\text{CO}_2(\text{aq})$).

Although the pre-bloom $\text{CO}_2(\text{aq})$ concentration may be as little as $15 \mu\text{Mol kg}^{-1}$, when $\text{CO}_2(\text{aq})$ is depleted it is quickly (within seconds (Zeebe and Wolf-Gladrow 2001)) replenished by conversion of HCO_3^- to $\text{CO}_2(\text{aq})$. Because of this rapid inter-conversion between different dissolved carbon forms, a phytoplankton drawdown of more than $15 \mu\text{Mol kg}^{-1}$ of carbon does not cause $\text{CO}_2(\text{aq})$ to run out. This can

only happen if all DIC is exhausted or if the pH is significantly changed. In reality DIC only rarely drops below $1800 \mu\text{Mol kg}^{-1}$. Despite the enormous uptake by phytoplankton, it is unlikely that $\text{CO}_2(\text{aq})$ ever falls much below about $10 \mu\text{Mol kg}^{-1}$ in the open ocean, even following intense spring blooms such as those that occur annually in the northeast North Atlantic (Tyrrell and Taylor 1995).

The preceding discussion suggests that carbon availability never sets a limit to the *final amount* of phytoplankton growth in a season, but it is possible that it sets a limit to the *instantaneous rate* of growth at the height of blooms. Despite the ever-presence of DIC, experimental evidence suggests that its rate of supply to cells can limit diatom growth rate during blooms (Riebesell et al. 2000). The hypothesis that CO_2 supply by calcification is important to coccolithophores implies that they should be especially successful at the height of and towards the end of spring blooms, when growth rates are high and $\text{CO}_2(\text{aq})$ concentrations relatively low. An objective analysis does suggest that most *E. huxleyi* blooms are associated with declining nitrate concentrations (Iglesias-Rodriguez et al. 2002), which must correlate with declining $\text{CO}_2(\text{aq})$. However, blooms (or at least bright waters) persist for many months after the spring blooms in the eastern Bering Sea (Sukhanova and Flint 1998; Iida et al. 2002) and elsewhere, into Jul/Aug/Sep when shortages of nitrate and phosphate rather than of $\text{CO}_2(\text{aq})$ are most likely to be critical for growth rate. In addition to physiological/biochemical evidence against this hypothesis (Rost and Riebesell, this volume) from the ecological point of view it appears that this hypothesis is not able to explain all aspects of *E. huxleyi* distribution.

High carbonate saturation state (carbonate ion concentration)

A wide range of experiments, mostly on other marine calcifiers but also on *E. huxleyi*, suggest a possibly important role for carbonate ion concentration (carbonate saturation state) in determining where blooms can occur.

Coral reefs are restricted to low latitudes, most probably because of a dependence on the carbonate saturation state of seawater (Ω) (Kleypas et al. 1999a), which attains highest values between about $0\text{--}30^\circ$ of latitude, and then falls away towards the poles (Fig. 5). Calcium carbonate saturation state is

$$\Omega = [\text{Ca}^{2+}] \cdot [\text{CO}_3^{2-}] / K'_{sp} \quad (2)$$

where K'_{sp} is the stoichiometric solubility product which takes different values for the different mineral phases aragonite (coral reefs, pteropods) and calcite (coccoliths, foraminifera shells) but for which the geographical trend is the same. Calcium concentration varies little throughout the ocean, so variability in Ω is mostly due to variability in CO_3^{2-} . Coral reefs today are found only where Ω is fairly high ($\Omega_{\text{arag}} > 3.0$ or so), and are generally vigorous and productive where Ω is par-

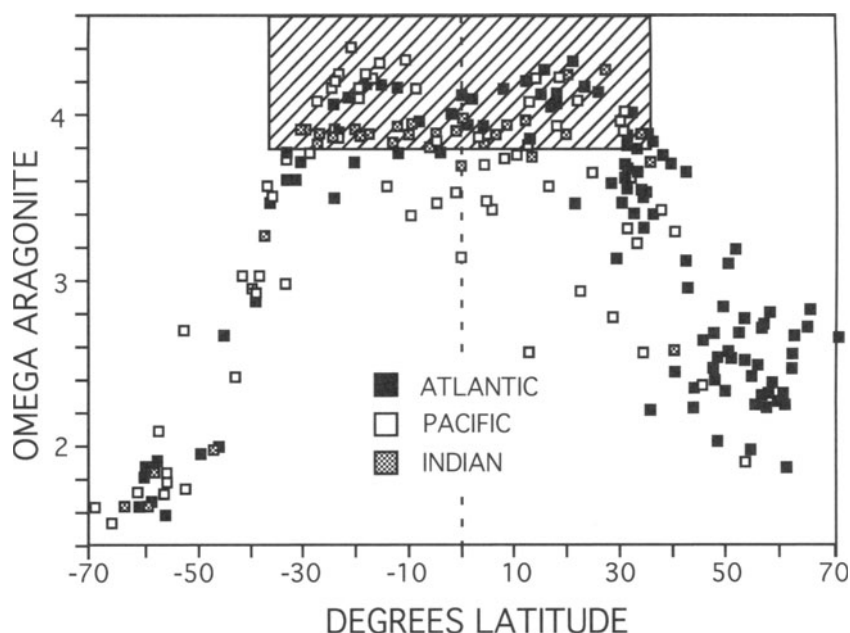


Fig. 5. Latitudinal distribution of aragonite saturation state in different oceans (taken from Opdyke and Wilkinson 1990)

ticularly high, for instance, the Bahamas, the Red Sea, and the coast of Papua New Guinea ($\Omega_{\text{arag}} > 3.9$ or so) (Kleypas et al. 1999b). Manipulation experiments on coral reefs and coralline algae have corroborated the importance of saturation state to coral reefs (references in Kleypas et al. 1999a). The coral reef biome within Biosphere II has been subjected to lowered Ω , again leading to reduced calcification (Langdon et al. 2000).

Although foraminifera exhibit a global distribution including polar waters (unlike reefs which are constrained to high- Ω waters) nevertheless they too respond to the saturation state of seawater. Laboratory experiments in which foraminifera have been grown at varying carbonate ion concentrations (varying Ω) have shown that the shell weight (thickness of the foram shells) is strongly dependent on the carbonate ion concentration (Spero et al. 1997). This was found both when TCO_2 was varied while alkalinity was kept constant, and also while alkalinity was varied while TCO_2 was kept constant (Spero et al. 1997). This effect, which is a precipitation effect and separate from dissolution effects related to carbonate ion concentration (Lohmann 1995), has recently been demonstrated graphically in the discovery of variation of foram shell weights over time. Barker and Elderfield (2002) recovered forams deposited well above the lysocline (so dissolution effects can be ignored) at various times back to the last glacial maximum and beyond. Forams deposited during the LGM, when carbonate ion concentration was higher because

of the lower atmospheric $p\text{CO}_2$, were found to be heavier than any found in the ocean today (Barker and Elderfield 2002).

This sensitivity of other marine calcifiers to carbonate ion concentration appears also to hold for coccolithophores. Coccolithophores (*G. oceanica* and *E. huxleyi*) in cultures, and also in field samples from the open ocean, were subjected to low Ω conditions, at which it was found that calcification rates declined and malformed coccoliths were produced (Riebesell et al. 2000; Rost and Riebesell this volume). The lowered calcification rates of Riebesell et al.'s experiments are somewhat hard to understand from a physiological point of view because *E. huxleyi* appears to obtain its carbon for coccoliths from bicarbonate (HCO_3^-), not from carbonate (CO_3^{2-}) (Paasche 2002). If this is correct then why should Ω be important to coccolithophores? However, corals are also thought to take up bicarbonate but yet they too are found experimentally to be sensitive to carbonate ion rather than bicarbonate ion concentrations (Marubini et al. 2001).

If further work substantiates this probable link between coccolithophore calcification and saturation state, this could be of interest beyond just the calculation of carbon fluxes during *E. huxleyi* blooms. The distribution of coral reefs in the ocean (where they can succeed in competition with sponges, seaweeds, etc) may be heavily influenced by geographical variations in Ω (Kleypas et al. 1999b). Perhaps the geographical distribution of Ω (Fig. 5) also affects where coccolithophores in general (and *E. huxleyi* blooms in particular) can occur (where *E. huxleyi* can compete successfully with other phytoplankton), not just their calcification rate. Perhaps the absence of coccolithophores from cold polar waters is because of difficulty in building sufficiently robust coccoliths at low Ω . In the same way that the geographical and temporal variation in success of diatoms is influenced by the abundance of silicate (e.g. Egge and Aksnes 1992) for diatom shell building, so too could the distribution of coccolithophores be influenced by geographical variation in the ease of coccolith building.

It is rather important to answer this question of sensitivity to carbonate ion because the emission of CO_2 into the atmosphere from burning of fossil fuels is, following diffusion of large amounts of the CO_2 into the surface ocean, rapidly acidifying the surface ocean and decreasing carbonate ion concentration and Ω there. It is estimated that Ω_{arag} averaged ~ 4.6 in the tropics 100 years ago, is currently ~ 4.0 , and is projected to drop to ~ 2.8 by 2100 (Kleypas et al. 1999a). Both coccolithophores and coral reefs could become substantial casualties of the rise in atmospheric and ocean carbon. We need to understand whether coccolithophores, coral reefs, & co. will be driven out of most or all oceanic environments by rising CO_2 and consequent falling CO_3^{2-} .

Type of grazers (microzooplankton and/or jellyfish)

As a top-down rather than a bottom-up forcing, the respective numbers of different species of grazers at a location may influence the viability of that location for different species of phytoplankton. The major grazers of *E. huxleyi* in the NE At-

lantic, as measured in 1991, were microzooplankton (Holligan et al. 1993a) and therefore variations in the density of microzooplankton could be important in determining where blooms of *E. huxleyi* can form.

Recently, Olson and Strom (2002) measured phytoplankton growth and microzooplankton grazing rates, by means of seawater dilution techniques inside and outside an *E. huxleyi* bloom area in the southeastern Bering Sea. They observed that "a reduced microzooplankton grazing is a key component in the formation and temporal persistence of *Emiliania huxleyi* bloom". Microzooplankton grazing seemed to shift from selective grazing on small phytoplankton cells outside the bloom to selective grazing on large cells (diatoms) inside the bloom. Reduced microzooplankton grazing of *E. huxleyi* than of photosynthetic dinoflagellates was also measured within a bloom off the Devon (UK) coast in July 1999 (Fileman et al. 2002), although not within a North Atlantic bloom in June 1991 where there was higher microzooplankton grazing of *E. huxleyi* than of the phytoplankton assemblage as a whole (Holligan et al. 1993a).

The Black Sea has seen large increases in the numbers of gelatinous carnivores (jellyfish: medusae *Aurelia aurita* and ctenophore *Mnemiopsis leidyi*) during the past two decades (Oguz et al. 2001), while during the last decade or so blooms of *E. huxleyi* have become a feature of the system (Cokacar et al. 2001). A similar scenario has been seen in the eastern Bering Sea, where a dramatic increase in jellyfish (dominated by the scyphozoan *Chrysaora melanaster*) was noted since the early 1990s (Brodeur et al. 2002). The peak of highest total biomass over the whole shelf was noted in 1997, the year of the first massive *E. huxleyi* blooms in the area. It is not clear whether the success of *E. huxleyi* is directly tied to jellyfish (via a trophic cascade). It appears from isotope ratios and stomach content analysis that *Chrysaora melanaster* have the same diet as juvenile pollock but that they also prey on macrozooplankton, in competition with adult pollock (Brodeur et al. 2002). Therefore, although it seems clear that jellyfish can depress zooplankton standing stocks, a direct connection of *E. huxleyi* appearance in 1997 with jellyfish increasing biomass remains to be proven.

In general, however, it needs to be kept in mind that it is intrinsically difficult to test hypotheses involving zooplankton or higher trophic levels due to a lack of sufficient understanding and data on their interactions with phytoplankton. Viruses specific for *E. huxleyi* may also play a role (Bratbak et al. 1996), but are likely to be more important in bloom termination than in bloom commencement (e.g. Wilson et al. 2002).

Other possible factors

In a review of the 'physiological ecology of marine coccolithophores', (Brand 1994), it was noted that *E. huxleyi* is likely to be *r*-selected rather than *K*-selected, since it has a high maximum growth rate (up to 2.8 doublings per day (Brand and Guillard 1981)). It was also suggested that *E. huxleyi* is usually found in cold waters, and in waters with low nutrient concentrations. Another review paper (Young 1994) suggested that there are three common features to the occurrence of placo-

lith-bearing coccolithophores such as *E. huxleyi*: firstly, they predominate in areas of upwelling; secondly, they are usually bloom-forming; and thirdly, they are normally dominant in coastal and shallow-sea assemblages.

Neither temperature nor salinity are likely to be significant causal factors. *E. huxleyi* is known to be one of the most eurythermal and euryhaline of species (Winter et al. 1994). Even though water temperature, in combination with other parameters, appears to be a good predictor of *E. huxleyi* blooms (Iglesias-Rodriguez et al. 2002) (they are associated with water temperatures between 3° and 15°C), this is probably due to secondary effects of temperature, such as its effect on water stability. “In general, blooms of *E. huxleyi* follow those of diatoms in waters that have been recently depleted in inorganic nutrients and are becoming more stable in terms of vertical mixing (e.g. following the relaxation of upwelling, or establishment of the seasonal thermocline)” (Holligan et al. 1993b). This precludes most very cold or very warm waters. A strong salinity control on *E. huxleyi* blooms is ruled out by the presence of blooms in both the open ocean (average about 35 ppt) and the semi-saline Black Sea (average about 18 ppt).

Although “seeding effects” (the advection of cells from elsewhere in order to provide the starting population that is required before a bloom can take place) have been considered important in the past (Birkenes and Braarud 1952), the case study of the eastern Bering Sea suggests otherwise. In that case enormous blooms ‘came from nowhere’ in the space of just two years (no visible bloom in 1995 or previous years, small bloom in 1996, enormous bloom in 1997) (Merico et al. 2003). The large blooms were able to develop rapidly from what must have been a small initial population. A time sequence of satellite images <http://www.soes.soton.ac.uk/staff/tt/eh/sequence.html> also shows that blooms can start all along the western coast of Norway at the same time, rather than one bloom seeding another to produce a “chain reaction” of blooms.

E. huxleyi has a requirement for thiamine (vitamin B₁) for growth, unlike many algae, but does not require vitamin B₁₂ (Carlucci and Bowes 1970). This may represent a further reason why *E. huxleyi* is not present during the first stages of spring blooms, but the ecological importance of vitamin B₁ has never been proven (Paasche 2002). *E. huxleyi* can grow well at low concentrations of iron, along with other open-ocean phytoplankton (Brand et al. 1983). However, this ability has not been shown to have any ecological significance, and *E. huxleyi* distribution is not at all correlated with regions of iron deficiency in the oceans.

Conclusions

Progress has been made towards uncovering the main environmental factors facilitating the development of *E. huxleyi* blooms. It seems unlikely that any single factor can explain all blooms. More probably a combination of conditions is required. High light and limiting silicate (to restrict diatoms) are probably essential, and high carbonate saturation state may also be critical, but much work remains to be done.

Modern satellites are excellent tools for mapping the distribution of *E. huxleyi* blooms, but the possibility of mimicking conditions shows the importance of in-situ verification. Annually repeating bright waters on the Patagonian Shelf particularly need to be sampled.

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Coccolithophores and the biological pump: responses to environmental changes

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Summary

Coccolithophores, which are considered to be the most productive calcifying organisms on earth, play an important role in the marine carbon cycle. The formation of calcite skeletons in the surface layer and their subsequent sinking to depth modifies upper-ocean alkalinity and directly affects air/sea CO₂ exchange. Recent work indicates that the productivity and distribution of coccolithophores are sensitive to CO₂-related changes in environmental conditions, both directly through acidification of surface seawater and indirectly through increasing upper-ocean thermal stratification. To assess possible responses of this group we examine the physiology and ecology of coccolithophores with regard to expected environmental changes. Potential feedbacks to atmospheric CO₂ increase, as could arise from changes in photosynthesis and calcification as well as from a shift in the dominance of coccolithophores, may be crucial when trying to forecast future climate change.

Introduction

The climate of the earth has undergone major changes over geologic time-scales. Climatic conditions and changes therein have immediate repercussions for the global biosphere, influencing the structure and productivity of ecosystems and the proliferation or disappearance of organisms. In turn, biological activity can exercise a direct impact on the climate. By driving many of the global elemental cycles, organisms can both mitigate or amplify climate change and may contribute, as suggested by the Gaia hypothesis (Lovelock 1979), to stabilising the climate. Global changes in environmental conditions, including climate conditions, are presently occurring at an unprecedented rate due to large-scale perturbations induced by human activities. While this is bound to give rise to ecosystem changes

on the global scale, we are only starting to understand the complex interplay between climate variability and ecosystem structure and functioning. Reliable prediction of the direction and magnitude of ecosystem changes, however, would be a prerequisite for assessing the risks involved in human-induced global change.

Despite a high degree of structural complexity, the energy flow and elemental cycling of many ecosystems are dominated by a comparatively small number of species. A first step in understanding possible consequences of environmental change on ecosystem functioning, therefore, is to determine the relevant responses of these key species. This also holds true for the marine pelagic system, the largest ecosystem on our planet. While the base of its complex food web is formed by more than 5000 species of marine phytoplankton, only a few taxonomic groups of phytoplankton, and within these groups a relatively small number of species, are responsible for most of the system's primary production, the energy transfer to higher trophic levels and the vertical export of biogenic material to the deep ocean. These key species can be further distinguished into so-called 'functional groups', i.e. phytoplankton building siliceous or calcareous shells, such as the diatoms and coccolithophores, respectively, flagellates forming organic plates or mucilaginous colonies, and cyanobacteria fixing atmospheric nitrogen. Each of these functional groups has a distinct effect on elemental fluxes, both between the surface and deep ocean as well as the overlying atmosphere.

With regard to the oceanic carbon cycle, the effect of calcifying plankton, including coccolithophores as the dominant calcifying phytoplankton, differs greatly from that of other primary producers (Fig. 1). The fixation of inorganic carbon via photosynthesis in the sunlit upper mixed layer and the vertical export of part of this organic material causes a draw down of CO_2 in the surface ocean. Remineralisation of the particulate organic carbon on its way to depth releases organically-bound CO_2 , which then accumulates in deeper layers. This process, termed the *organic carbon pump*, thereby causes a net draw down of CO_2 from the atmosphere into the ocean. In contrast, the production and export of calcium carbonate has the opposite effect on air/sea CO_2 exchange, causing a net release of CO_2 to the atmosphere. Due to its counteracting effect on CO_2 flux, this process is often referred to as the *carbonate counter pump* (while CO_2 release through carbon fixation may appear *counter-intuitive*, the intricacies of the underlying carbonate chemistry will be outlined below). The relative strength of the two biological carbon pumps, represented by the so-called *rain ratio* (the ratio of particulate inorganic to organic carbon in exported biogenic matter), to a large extent determines the flux of CO_2 between the surface ocean and the overlying atmosphere.

Recent work indicates that changes in seawater carbonate chemistry caused by rising atmospheric CO_2 (Fig. 2) can decrease biologically-mediated calcification (Gattuso et al. 1998; Wolf-Gladrow et al. 1999; Riebesell et al. 2000a; Zondervan et al. 2001). Changes in seawater CO_2 concentration and/or CO_2 -related changes of the carbonate system are also likely to modify phytoplankton species composition (Tortell et al. 2002), and may even alter the relative abundance of calcifying versus non-calcifying phytoplankton (Rost et al. 2003). Since changes in atmospheric pCO_2 give rise to corresponding changes in the carbonate system of surface

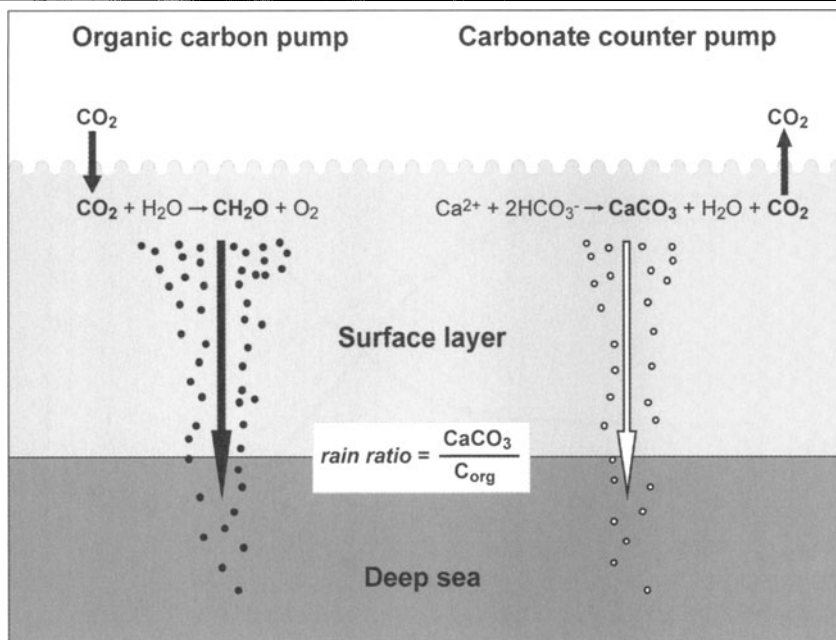


Fig. 1. The biological carbon pumps: Photosynthetic production of organic matter in the surface layer and its subsequent transport to depth, termed organic carbon pump, generates a CO_2 sink in the ocean. In contrast, calcium carbonate production and its transport to depth, referred to as the calcium carbonate pump, releases CO_2 in the surface layer. The relative strengths of these two processes (*rain ratio*) largely determine the biologically-mediated ocean atmosphere CO_2 exchange.

seawater with a time lag of less than one year (Zeebe and Wolf-Gladrow 2001), biological responses may occur in phase with the present rise in atmospheric CO_2 . Moreover, expected climate-induced changes in surface-ocean temperature, stratification, and mixing, and the related changes in mixed layer light conditions and nutrient cycling (Fig. 3), are bound to affect the structure and composition of marine pelagic ecosystems. These changes will have profound effects on the biological carbon pumps, both in absolute terms and with respect to their relative strengths.

In view of the rapid changes in environmental conditions presently occurring on a global scale, a major challenge in earth system sciences is to predict biospheric responses to global change. Any attempt to forecast biologically-mediated changes in marine biogeochemical cycling critically depends on our ability to explain the distribution and succession of the dominant phytoplankton groups in relation to biotic and abiotic environmental conditions. At present, we are far from understanding phytoplankton succession and distribution in today's ocean, let alone possible modifications therein in response to environmental change. In this

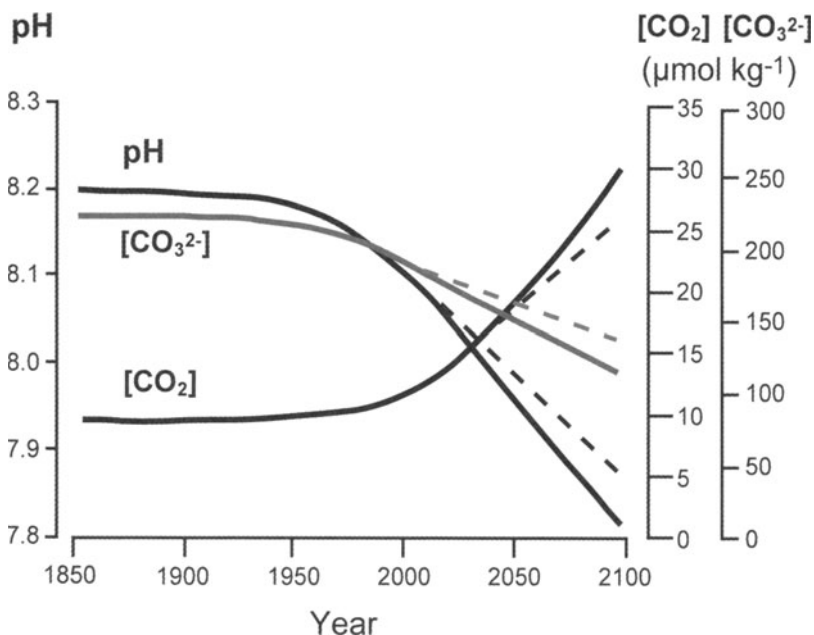


Fig. 2. Seawater pH and the dissolved carbon dioxide (CO_2) and carbonate ion (CO_3^{2-}) concentrations in the surface layer of the ocean assuming a “business as usual” (IS92a) anthropogenic CO_2 emission scenario (Houghton et al. 1995). Dashed lines represent the predicted changes in carbonate chemistry if CO_2 emissions are reduced according to the Kyoto Protocol (modified after Wolf-Gladrow et al. 1999).

chapter we will outline some of the relevant characteristics of coccolithophores in the framework of environmental change and how these may affect the competitive advantage of this group in the future. While the focus of this paper will be on recent natural and anthropogenic global change, we will also look for possible indications in the geological past and will examine potential consequences for biogeochemical cycling in the ocean in the future.

Biogeochemical role of coccolithophores

Coccolithophores are a comparatively young group of planktic microalgae, which first appeared in the late Triassic about 200 Mya (million years ago; Bown et al. this volume) and started to produce conspicuous accumulations of calcium carbonate in the rock record since the late Jurassic (ca. 150 Mya) (Morse and Mackenzie 1990). From this point on, coccolithophores have had a major impact on marine biogeochemical cycling with tremendous consequences for earth's climate. Together with planktic foraminifera, which became prominent in the

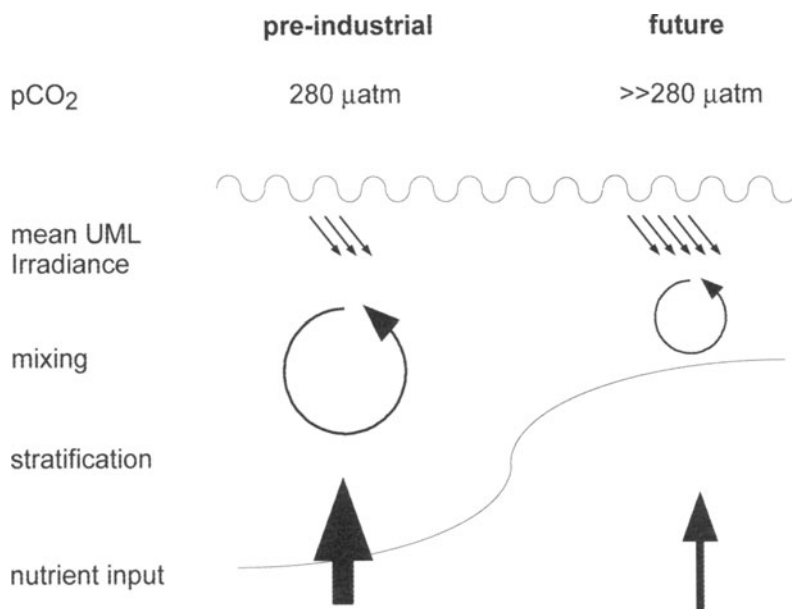
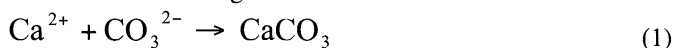


Fig. 3. The progressive increase in atmospheric CO₂ affects the marine biota in various ways: directly through changes in surface ocean chemistry (see Fig. 2) and indirectly through rising mean global temperatures causing increased surface ocean stratification. This in turn reduces the nutrient input from deeper layers and increases the light availability due to shoaling of the upper mixed layer (UML).

Middle Cretaceous (ca. 100 Mya), coccolithophores are mainly responsible for creating and maintaining the ocean's vertical gradient in seawater alkalinity. The coupling between calcification and photosynthesis in coccolithophores, and to some extent also in the symbiont-bearing foraminifera, implies that large-scale calcification is concentrated in the photic zone of the ocean. CaCO₃ formation not only binds dissolved carbon into particulate carbon and thereby reduces total dissolved inorganic carbon, it also lowers seawater alkalinity and changes the equilibrium between the different forms of dissolved inorganic carbon. The draw down and vertical flux of CaCO₃-bound inorganic carbon and alkalinity drives the carbonate counter pump and consequently causes an increase in atmospheric pCO₂.

But how can a process transporting inorganic carbon from the surface to the deep ocean be responsible for maintaining high atmospheric pCO₂? The formation of one unit of calcium carbonate according to



lowers the dissolved inorganic carbon (DIC) concentration, with

$$\text{DIC} = [\text{CO}_2] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}] \quad (2)$$

by one unit. Due to its double negative charge the concentration of carbonate ions carries the factor 2 in the expression of total alkalinity (*Talk*), with

$$\text{Talk} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B(OH)}_4^-] + [\text{OH}^-] - [\text{H}^+] \quad (3)$$

Thus, the precipitation of one unit of calcium carbonate lowers seawater alkalinity by two units. How this affects the concentration of dissolved CO_2 is best illustrated, following Zondervan et al. (2001), by plotting CO_2 concentration as a function of DIC and *Talk* (Fig. 4). Starting at typical surface ocean conditions of $\text{DIC} = 2.0 \text{ mmol kg}^{-1}$ and $\text{Talk} = 2.3 \text{ mmol kg}^{-1}$ (beginning of diagonal vector on left side), the corresponding CO_2 concentration, CO_2 , is $10.5 \text{ } \mu\text{mol kg}^{-1}$ (assuming $T=15^\circ\text{C}$, $S=35$). Precipitating $50 \text{ } \mu\text{mol kg}^{-1}$ of CaCO_3 lowers DIC and *Talk* by 0.05 and 0.1 mmol kg^{-1} , respectively. With the new DIC and *Talk* values of 1.95 mmol kg^{-1} and 2.2 mmol kg^{-1} (end of diagonal vector), the corresponding CO_2 concentration now is $12.4 \text{ } \mu\text{mol kg}^{-1}$. Assuming that the seawater initially was in equilibrium with the atmosphere with respect to CO_2 , it is now CO_2 over-saturated. To restore equilibrium, 0.63 units of CO_2 need to be released for each unit of inorganic carbon precipitated via calcification. In our example, $50 \text{ } \mu\text{mol kg}^{-1}$ CaCO_3 production would require a degassing of $32 \text{ } \mu\text{mol kg}^{-1}$ of CO_2 in order to maintain CO_2 equilibrium between our parcel of seawater and the overlying atmosphere. Due to the decrease in the buffer capacity with increasing CO_2 (decreasing pH) of seawater, the ratio of CO_2 released per carbonate precipitated increases with rising atmospheric CO_2 (Frankignoulle et al. 1994). Under conditions expected for the year 2100 (see Fig. 2), the surface ocean equilibrium CO_2 concentration would be ca. $27 \text{ } \mu\text{mol kg}^{-1}$. Under these conditions, precipitation of $50 \text{ } \mu\text{mol kg}^{-1}$ CaCO_3 would release ca. $40 \text{ } \mu\text{mol kg}^{-1}$ of CO_2 , corresponding to 0.79 units of CO_2 for each unit of CaCO_3 (right vectors). If global calcification were to remain constant over this time, increased calcification-induced CO_2 release results in a positive feedback to rising atmospheric CO_2 (Zondervan et al. 2001).

Aside from their predominant role in global ocean calcification, coccolithophores are also important primary producers. Their geographical distribution ranges from oligotrophic subtropical gyres to temperate and high latitude eutrophic regimes. Representatives of this group equally thrive in low chlorophyll recycling systems as well as in high biomass new production systems. Of the ca. 200 species of coccolithophores, however, only very few species form intense blooms over large areas of the ocean (Tyrrell and Merico this volume). Among these, *Emiliania huxleyi* and *Gephyrocapsa oceanica* are by far the most prominent bloom-formers. As calcifying primary producers, coccolithophores contribute to both the *organic carbon pump* and the *carbonate counter pump*. The draw down of CO_2 due to organic carbon production is thereby partly compensated by the release of CO_2 via calcification. Primary production by coccolithophores is therefore a smaller sink for CO_2 when compared to a non-calcifying production system, such as a diatom bloom (Robertson et al. 1994). Due to calcite overproduction, a phenomenon typical for *Emiliania huxleyi* when growing into nutrient limitation, blooms of this species can even turn into a net source for CO_2 to the atmosphere (Purdie and Finch 1994). Obviously, the ratio of coccolithophore calcification to organic matter production, but even more so the ratio of calcareous to non-

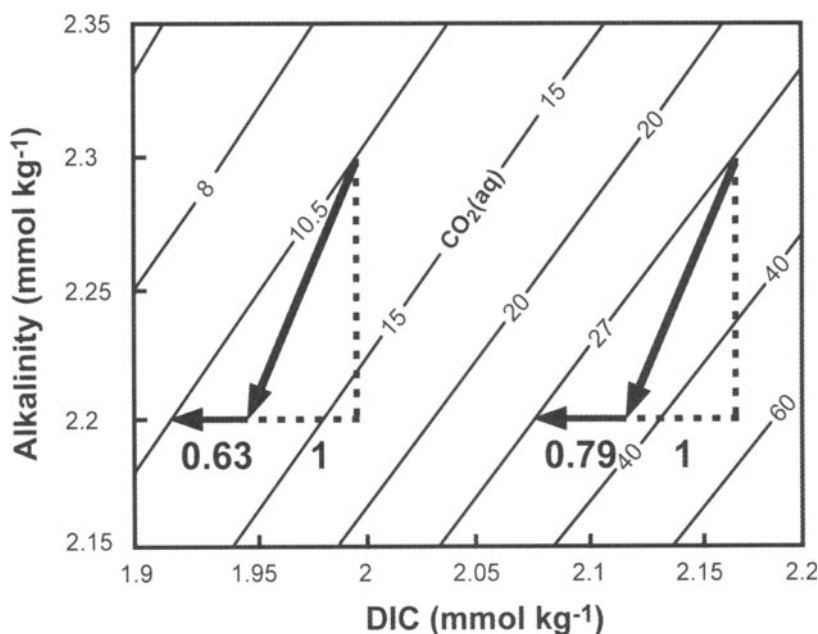


Fig. 4. Changes in the concentrations of DIC, total alkalinity, and CO_2 as a result of calcification at pre-industrial times (left vectors) and in year 2100 (right vectors). Numbers at the vectors are relative values. The difference in the effect of calcification on seawater carbonate chemistry in the two scenarios results from a CO_2 -related change in the seawater buffer capacity (see text for details, modified after Zondervan et al. 2001).

calcareous primary production, strongly determine the relative strengths of the two biological carbon pumps. CaCO_3 may also act as a “ballast” mineral that increases the transfer efficiency of POC from the surface to the deep sea (Armstrong et al. 2002). The presence of coccoliths could thus enhance the sedimentation of POC such as in faecal pellets (Buitenhuis et al. 1996). According to this “ballast” hypothesis variations in the rain ratio would have been generally smaller than previously assumed (Armstrong et al. 2002; Klaas and Archer 2002).

On geological time-scales variation in coccolith abundance in the sedimentary record is often correlated with glacial-interglacial transitions, indicating a predominance of coccolithophores during interglacial periods (McIntyre et al. 1972; Henrich 1989). The presumably lower contribution of coccolithophores relative to non-calcifying phytoplankton may have contributed to the lower atmospheric CO_2 levels in glacial times (Archer and Maier-Reimer 1994; Harrison 2000; Ridgwell et al. 2002). A large-scale effect of present climate forcing on the relative contribution of calcareous to primary production is indicated by a basin-wide shift in the composition of sedimenting particles in the North Atlantic (Antia et al. 2001). The

observed steady decrease in the opal:carbonate ratio since the early eighties may reflect an increase in the abundance and productivity of coccolithophores relative to diatoms. Changes in environmental conditions related to climate forcing are likely to affect phytoplankton functional groups differently (Boyd and Doney 2002). Forecasting the response of marine ecosystems to global change therefore requires an understanding of the factors that determine the distribution and productivity of key phytoplankton groups. A first step to assessing the sensitivity of coccolithophores to environmental change will be to examine the physiology and ecology of this group in relation to other dominant phytoplankton taxa.

Coccolithophore ecophysiology

Most of our information about the physiology and ecology of coccolithophores originates from the extensive work on *Emiliania huxleyi*, a species that is somewhat unusual for the group of coccolithophores. It has evolved only 270.000 years ago from the older genus *Gephyrocapsa* (Thierstein et al. 1977), became dominant 70.000 years ago and is now the most abundant coccolithophore in the ocean. Only few coccolithophores form blooms like *E. huxleyi*, thus *E. huxleyi* is more r-selected (i.e. pioneer) than most other coccolithophores. Other specialities are the phenomenon of coccolith-overproduction and the lack of light-inhibition in this species. For an extensive review on *E. huxleyi* we refer to Paasche et al. (2002). In view of the current increase in atmospheric $p\text{CO}_2$, coccolithophores could be affected in various ways: directly through changes in surface ocean carbonate chemistry (Fig. 2), and indirectly through rising mean global temperatures causing increased surface ocean stratification with respective changes in the light regime and nutrient supply (Fig. 3). In the following sections on photosynthesis and calcification we will therefore focus on the effect of light, inorganic carbon and other nutrients. Furthermore, possible interactions between calcification and photosynthesis will be discussed.

Photosynthesis and growth

Photosynthesis involves a series of reactions that start with capturing light energy, transferring it into the energy-conserving compounds NADPH and ATP, and using these compounds to fix CO_2 in the Calvin cycle. Actively growing algae allocate about 50% of recent photosynthate to protein synthesis, resulting in a competition for NADPH and ATP between CO_2 fixation, transport processes, nitrate reduction and protein formation (Geider and MacIntyre 2001). Thus photosynthesis and downstream processes leading to cellular growth are primarily light-dependent processes. Photosynthesis-irradiance response curves are commonly used to assess the photosynthetic performance. Parameters derived from these curves such as the light-saturated photosynthetic rate (P_m), light-limited initial slope (α), maximum quantum yield (Φ_m) as well as Chl *a*:C ratios are similar for

E. huxleyi to those of other phytoplankton species (Nielsen 1997). Yet, with light-saturation irradiances (E_k) between 70 and 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (e.g. Paasche 1967; Van Bleijswijk et al. 1994; Nielsen 1997), *E. huxleyi* reaches saturation levels at irradiances generally higher than those found for diatoms or dinoflagellates (Richardson et al. 1983). Moreover, elevated irradiances have no or little inhibitory effect on photosynthesis of *E. huxleyi*, which has been verified for irradiances up to 1700 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (e.g. Nielsen 1995; Nanninga and Tyrrell 1996). The tolerance of *E. huxleyi* to high light intensities is also reflected in its predominance during times when surface waters are highly stratified in the summer (Nanninga and Tyrrell 1996; Tyrrell and Taylor 1996).

In the dark reaction of photosynthesis CO_2 is fixed by the carboxylating enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). Due to the poor substrate affinity of RubisCO for CO_2 (K_M 20–70 $\mu\text{mol L}^{-1}$, Badger et al. 1998) and the low CO_2 concentrations present in seawater (5–25 $\mu\text{mol L}^{-1}$) photosynthesis of phytoplankton potentially suffers from CO_2 limitation. To overcome the imperfection of their main carboxylating enzyme, most microalgae have developed mechanisms to enhance the CO_2 concentration at the site of carboxylation. These CO_2 concentrating mechanisms (CCMs) comprise active uptake of CO_2 and/or HCO_3^- into the algal cell and/or the chloroplast. Also involved in most CCMs is the enzyme carbonic anhydrase (CA), which accelerates the otherwise slow rate of conversion between HCO_3^- and CO_2 . Owing to the operation of CCMs, most marine phytoplankton have high affinities for inorganic carbon and reach photosynthetic carbon saturation under ambient CO_2 levels (Raven and Johnston 1991).

Unlike other dominant phytoplankton groups *Emiliania huxleyi* has comparatively low affinities for inorganic carbon and photosynthesis appears to be carbon-limited in today's ocean (e.g. Paasche 1964; Sikes and Wheeler 1982; Nielsen 1995). This has led to the speculation that this species may rely entirely on diffusive CO_2 supply for photosynthesis (Raven and Johnston 1991). Recent studies, however, provide clear evidence for active carbon uptake in *E. huxleyi* (e.g. Nimer and Merret 1996; Rost et al. 2002, 2003). The efficiency of the CCM employed by *E. huxleyi* appears to be low when compared to other phytoplankton taxa (e.g. Burkhardt et al. 2001; Rost et al. 2003; Fig. 5). In view of close coupling between photosynthesis and calcification it has been hypothesized that *E. huxleyi* is able to use HCO_3^- for photosynthesis through the process of calcification (Sikes et al. 1980; Nimer and Merret 1993), potentially representing a cost-effective alternative to the classical CO_2 concentrating mechanism (Anning et al. 1996). The potential role of calcification for photosynthetic carbon acquisition will be dealt with in the section on calcification below.

In contrast to photosynthetic carbon fixation, cell division rate of *E. huxleyi* is not limited by DIC under natural conditions, thus it does not increase under elevated CO_2 or DIC concentrations (e.g. Paasche et al. 1996; Buitenhuis et al. 1999; Clark and Flynn 2000; Riebesell et al. 2000b). The "mismatch" between cell division and carbon fixation can cause large variations of carbon cell quota, with low values under sub-saturating irradiances and DIC concentrations, and high values at

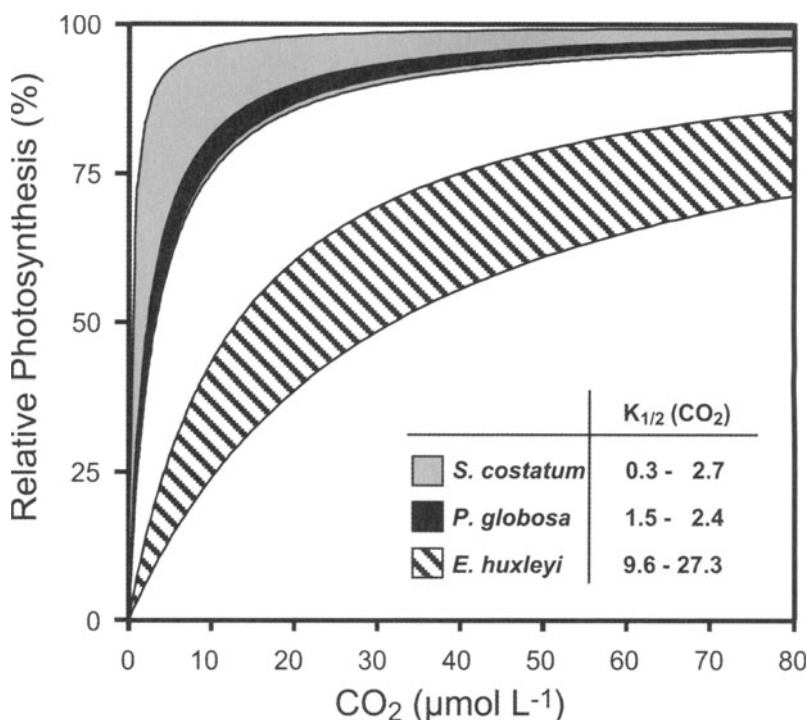


Fig. 5. Photosynthesis of phytoplankton species differs with respect to CO_2 sensitivity: While most species (here *Skeletonema costatum* and *Phaeocystis globosa*) are at or close to CO_2 saturation at present day CO_2 levels ($8\text{--}20 \mu\text{mol L}^{-1}$), coccolithophores such as *E. huxleyi* have comparatively low affinities for inorganic carbon and appear to be carbon-limited in today's ocean. This raises the possibility that coccolithophores may benefit directly from the present increase in atmospheric CO_2 . The range in half-saturation concentrations ($K_{1/2}$; in $\mu\text{mol L}^{-1}$) for photosynthesis shown here reflects the degree of regulation as a function of pCO_2 during growth (according to Rost et al. 2003). Highest apparent affinities for CO_2 were generally observed in cells which were grown under low pCO_2 .

satürating irradiances and DIC concentrations (e.g. Van Bleijswijk et al. 1994; Paasche 1999; Zondervan et al. 2002). The variability in carbon quota is even higher when cell division of *E. huxleyi* is limited by phosphorus or nitrogen (Paasche 1998; Riegman et al. 2000). The observed high flexibility in its carbon quota may be of ecological relevance, providing a mechanism to maintain high growth rates under limiting conditions. This could reduce constrictions otherwise imposed by the low affinity carbon acquisition.

In addition to light and carbon, other resources such as phosphorus and nitrogen as well as trace elements are required for the functioning of the photosynthetic apparatus and effective growth. *E. huxleyi* has extremely high affinity phosphate uptake systems with half-saturation constants as low as 1 nM (Riegman et al. 1992, 2000). A constitutive and an inducible alkaline phosphatase enables the cells to also use organic phosphate esters at nanomolar levels. Despite the ability of *E. huxleyi* to accumulate surplus phosphate to some degree, luxury consumption for phosphate is rather low relative to other species. With regard to nitrate utilisation, *E. huxleyi* was not found to be an exceptional competitor under N-limitation (Riegman et al. 1992, 2000). Maximum uptake rates are fairly low and the half-saturation constant of 0.2 μM is similar or higher than those of other algae (Page et al. 1999; Riegman et al. 2000). Some strains of *E. huxleyi* can use other N-compounds such as amino acids (Ietswaart et al. 1994), low molecular weight amides, urea, and purines (Palenik and Henson 1997). As in the case of phosphate, luxury consumption for nitrate is low in *E. huxleyi* compared to other phytoplankton taxa, such as diatoms (Grime 1979; Raven 1997). Iron and zinc requirements of *E. huxleyi* are lower than of coastal diatoms but comparable with those of other oceanic phytoplankton species (Brand 1991; Sunda and Huntsmann 1995; Muggli and Harrison 1996; Morel et al. 1994). Owing to the exceptionally high affinity for phosphate as well as its ability to use organic phosphate esters, however, *E. huxleyi* can outcompete other phytoplankton species under elevated N:P ratios (Riegman et al. 1992, 2000).

Calcification and its relation to photosynthesis

Despite intensive research on coccolithophores the function of calcification is still not completely understood (Young 1994; Brownlee et al. 1994; Paasche 2002; Brownlee and Taylor this volume). It has been hypothesized that the cell-covering layer of coccoliths, the coccosphere, serves to protect against grazing and/or virus attack and that coccoliths could bundle and modify the light intercepted by the algal cell (Young 1994). While these hypotheses amongst various others remain speculative, there is some support for the “trash-can function” of calcification, whereby CaCO_3 precipitation serves as a mechanism to facilitate the use of bicarbonate in photosynthesis (see below). The ratio of calcification to photosynthesis in coccolithophores, in the literature often referred to as the C/P or PIC/POC ratio, has recently received much attention as it provides information on the carbon metabolism of coccolithophores and bears implications for the *rain ratio*. In the following the light-, carbon- and nutrient-dependence of calcification will be discussed in comparison to photosynthesis, with emphasis on respective changes in the PIC/POC ratio.

Calcification of coccolithophores is predominantly a light-dependent process. This has been shown in numerous incubation experiments for *E. huxleyi* (e.g. Paasche 1962, 1964, 1965, 1966; Sikes et al. 1980; Linschooten et al. 1991) as well as *Coccolithus pelagicus* (Paasche 1969) and has been verified also under natural conditions in mesocosm and shipboard experiments (e.g. Van der Wal et

al. 1995; Balch et al. 1992; Holligan et al. 1993). Due to the different light-dependence of calcification compared to photosynthesis, the PIC/POC ratio in *E. huxleyi* generally decreases towards low growth irradiances (e.g. Van Bleijswijk et al. 1994; Balch et al. 1996; Paasche 1999; Zondervan et al. 2002). Balch et al. (1996) suggested that this light-dependence in PIC/POC offers an ecological adaptation strategy. Ceasing calcification under extreme light-limitation, as it occurs at the base of the photic zone, lowers cell density and thus may reduce the loss of cells through sinking below the photic zone. Since calcification continues when growth is limited by nutrient deficiency it could also provide a means of dissipating absorbed light energy to avoid photo-damage under nutrient limitation (Paasche 2002).

Both calcification and photosynthesis draw carbon from the large pool of dissolved inorganic carbon in seawater. While for photosynthesis, both CO_2 and HCO_3^- are used in variable proportion, for calcification, HCO_3^- is thought to be the main or only carbon source (Paasche 1964; Sikes et al. 1980). A predominantly HCO_3^- based calcification is indicated by the $\delta^{13}\text{C}$ composition of the coccoliths, which is close to the $\delta^{13}\text{C}$ of HCO_3^- (Sikes and Wilbur 1982; Rost et al. 2002). Increasing DIC was found to increase the rate of calcification in *E. huxleyi* (Paasche 1964; Buitenhuis et al. 1999; Berry et al. 2002). While elevated CO_2 concentrations under constant DIC have been shown to reduce calcification in light-saturated cultures of *E. huxleyi* and *G. oceanica* (Riebesell et al. 2000a), this trend in calcification was not observed under light-limitation (Zondervan et al. 2002). Under N- and P-limitation the responses in calcification to different CO_2 or DIC concentrations seems more pronounced than in nutrient replete cultures. Berry et al. (2002) investigated a low calcifying strain of *E. huxleyi* and observed a stimulation in the rate of calcification upon DIC enrichment (constant pH) as well as under decreasing CO_2 concentrations (increasing pH). Due to different responses of calcification and photosynthesis to changes in carbonate chemistry, the PIC/POC ratio in *E. huxleyi* generally increases with decreasing CO_2 or increasing DIC concentrations (Paasche 1964; Riebesell et al. 2000a; Berry et al. 2002; Zondervan et al. 2002; Fig. 6). This trend persists over a range of irradiances and only weakens under severe light limitation. Most of this change is caused by the response in photosynthesis, which is generally more sensitive to changes in CO_2 and DIC concentration than calcification. Phosphorus and nitrogen limitation was stated to stimulate calcification (e.g. Riegman et al. 2000; Berry et al. 2002), which is often deduced from the observed increase in the PIC/POC ratio upon nutrient limitation. While under nutrient replete conditions PIC/POC ratios are generally below unity, under P- and N-limitation values up to 2.3 have been observed (cf. Paasche 2002). This led to the suggestion that calcification plays a role in nutrient acquisition such as phosphorus metabolism. In fact, *E. huxleyi* has the highest affinity for phosphate of all investigated phytoplankton species (Riegman et al. 2000). Moreover, phosphorus deficiency induced recalcification of a non-calcifying strain of *E. huxleyi* (Paasche 1998). High PIC/POC ratios under low nutrient conditions may in some cases, however, simply reflect the lower nutrient dependency of calcification compared to photosynthesis. Since coccolith forma-

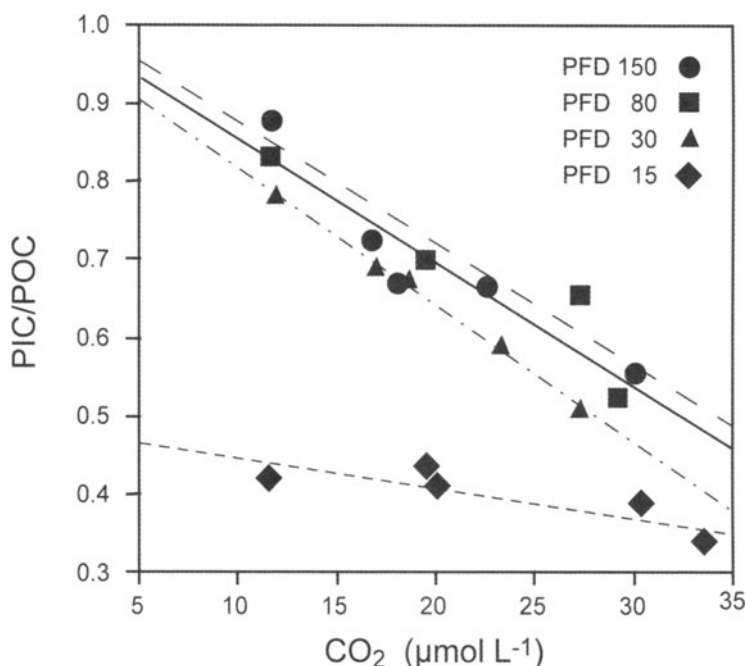
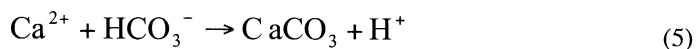
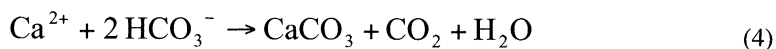


Fig. 6. Rising CO₂ decreases the ratio of calcification to organic carbon production (PIC/POC) in *E. huxleyi*. The decrease in PIC/POC is caused by enhanced photosynthetic carbon fixation and reduced or constant calcification. This trend is consistent over a range of photon flux densities (PFDs; in μmol photons m⁻² s⁻¹), yet declines under severe light-limitation (modified after Zondervan et al. 2002).

tion is predominantly a light-dependent process any kind of nutrient limitation affecting the rate of photosynthesis is bound to increase the PIC/POC ratio. This effect was also observed for growth under zinc-limitation (K. Schulz, pers. com.).

To what extent the two cellular processes calcification and photosynthesis are directly coupled is still an open question. While photosynthesis obviously drives calcification by providing the energy required to transport inorganic carbon and calcium ions and to accumulate them inside the coccolith producing vesicles, the question whether calcification is also beneficial for photosynthesis is still unresolved (Brownlee and Taylor this volume). Provided that HCO₃⁻ is the carbon source for calcification, one possible benefit for photosynthesis could be the release of CO₂ or protons in the course of calcification according to the following reactions:



Either CO_2 could be used directly in photosynthesis or protons could be used in the conversion of HCO_3^- to CO_2 . In either case this would provide the calcifying cell with a mechanism to access HCO_3^- , which represents the largest pool of inorganic carbon in seawater.

A functional coupling between calcification and photosynthesis has first been proposed by Sikes et al. (1980) and has since been favoured by various authors (e.g. Nimer and Merret 1993; Brownlee et al. 1994; Anning et al. 1996; Buitenhuis et al. 1999). While compelling evidence for or against such a mechanism is still lacking, it appears that calcification in coccolithophores is neither a prerequisite for efficient photosynthesis nor is it particularly effective in mitigating CO_2 limitation. This is indicated, for example, by the fact that photosynthesis continues unaltered when calcification ceases, such as when cells are grown in calcium-free medium (Paasche 1964). Likewise, non-calcifying cells can photosynthesise as efficiently as, or even more efficiently than, calcifying ones (Fig. 7). Moreover, mass spectrometric measurements indicate that non-calcifying cells of *E. huxleyi* are capable of direct uptake of HCO_3^- , which implies that HCO_3^- utilisation is not tied to calcification. That calcification also appears unsuited to prevent CO_2 limitation of coccolithophores is indicated by observations that the rate of photosynthesis decreases with decreasing CO_2 concentration despite a concomitant increase in calcification rate (Riebesell et al. 2000a; Berry et al. 2002; Zondervan et al. 2002). In essence, if supply of CO_2 for photosynthesis is indeed a primary role of calcification, this mechanism is rather inefficient when compared to CO_2 concentrating mechanisms of non-calcifying phytoplankton such as diatoms and *Phaeocystis* (Burkhardt et al. 2001; Rost et al. 2003).

Despite a wealth of information on the effect of environmental conditions on coccolith production, the mechanism of calcification by coccolithophores is not completely understood (Brownlee and Taylor this volume). Unlike other calcifying organisms, where calcification occurs in the extracellular space, calcite precipitation in coccolithophores takes place in intracellular vesicles and hence is under complete control of the cell. It therefore comes somewhat as a surprise that calcification of coccolithophores shows a similar dependency on seawater carbonate chemistry as in foraminifera and corals (Gattuso et al. 1998; Wolf-Gladrow et al. 1999; Riebesell et al. 2000a; Zondervan et al. 2001). It is still uncertain which is the predominant carbon species used for calcification and which parameters, seawater pH, DIC, CO_2 , HCO_3^- or CO_3^{2-} concentration ultimately has the strongest effect on biogenic calcification. For practical purposes a reasonable representation of the effect of carbonate chemistry on biologically-mediated CaCO_3 precipitation can be based on the carbonate saturation state of seawater (Ω) which is expressed as:

$$\Omega = \frac{[\text{Ca}^{2+}]_{\text{sw}} \times [\text{CO}_3^{2-}]_{\text{sw}}}{K_{\text{sp}}^*} \quad (6)$$

where $\text{Ca}_{\text{sw}}^{2+}$ and $\text{CO}_{3\text{sw}}^{2-}$ are the calcium and carbonate ion concentrations in seawater and K_{sp}^* is the stoichiometric solubility product, defined as $K_{\text{sp}}^* = \text{Ca}_{\text{sat}}^{2+} \times$

CO_3^{2-} sat. Here Ca^{2+} sat and CO_3^{2-} sat refer to the equilibrium ion concentrations in a seawater solution saturated with CaCO_3 .

That the carbonate saturation state may be the relevant parameter with regard to possible dependencies of biogenic calcification on seawater chemistry becomes evident when taking a look at the geological past. Recent evidence from fluid inclusions suggests, however, that calcium concentrations in the Cretaceous were more than twice as high as in the modern ocean (Horita et al. 2002). As CaCO_3 compensation tends to restore the saturation state of the ocean towards CaCO_3 saturation, higher calcium concentrations would correspond to lower carbonate ion

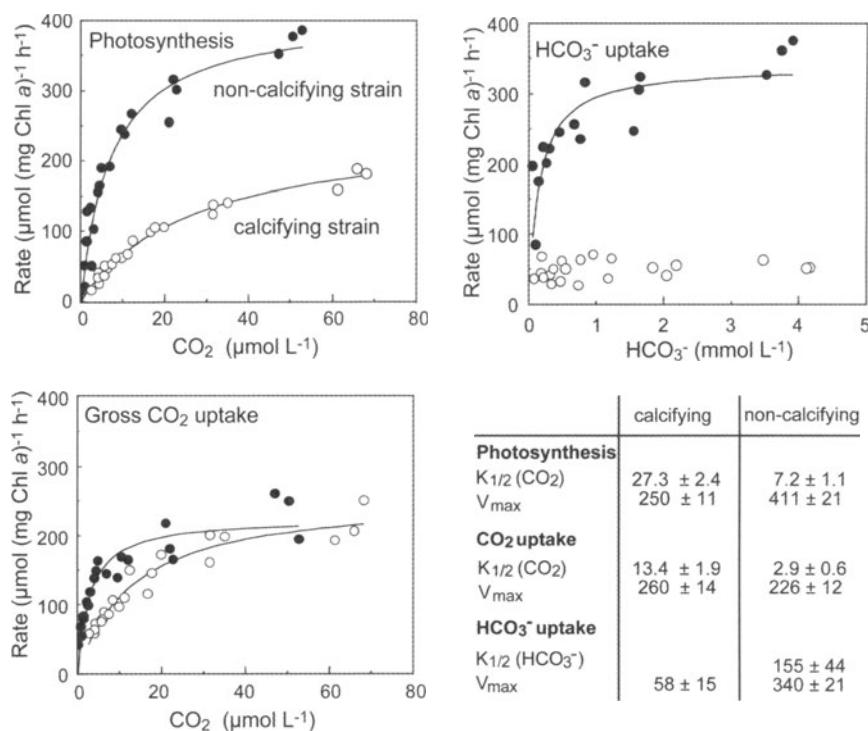


Fig. 7. Comparison of net photosynthesis, gross CO_2 uptake, and HCO_3^- uptake between a calcifying strain (PML B92/11) and a non-calcifying strain of *E. huxleyi* (obtained from Dr. Elzenga, University of Groningen). Half saturation concentrations ($K_{1/2}$) and maximum rates (V_{\max}) are given in units of $\mu\text{mol L}^{-1}$ and $\mu\text{mol (mg Chl } a)^{-1} \text{ h}^{-1}$, respectively. Culture conditions and measurements as in Rost et al. (2003), cells were acclimated to 360 ppmv CO_2 ($\sim 14 \mu\text{mol L}^{-1}$). Mass spectrometric procedures according to Badger et al. (1994) allow to estimate both CO_2 and HCO_3^- uptake by utilizing the chemical disequilibrium which exists between CO_2 and HCO_3^- during photosynthesis. The non-calcifying strain photosynthesises more efficiently than the calcifying one. Moreover, the non-calcifying cells of *E. huxleyi* are capable of direct uptake of HCO_3^- , which implies that HCO_3^- utilisation is not tied to calcification.

concentrations. In spite of lower expected CO_3^{2-} concentration, massive accumulation of biogenic carbonates occurred in the Cretaceous, suggesting that the carbonate saturation state may exert a stronger control on biogenic calcification than any of the other possible candidates, e.g. pH, CO_2 , or CO_3^{2-} concentrations.

As an interesting side-line we note that the evolutionary development and proliferation of the two dominant pelagic calcifiers, coccolithophores and foraminifera, coincides with a period of high seawater calcium concentrations. All living organisms use calcium in signal transduction and hence need to keep intracellular calcium at extremely low levels. If higher than present seawater calcium concentrations were indeed detrimental to marine organisms, calcification may provide an efficient way of maintaining the balance between high external and low intracellular calcium concentrations. In such a scenario, the accumulation of massive coccolith chinks in the late Cretaceous, which may have contributed to the concomitant rapid decline in seawater calcium concentrations (Volk 1989), would have improved living conditions in the marine environment. Whether or not calcite precipitation has provided calcifying organisms with a competitive advantage over non-calcifiers under elevated calcium concentrations remains to be tested.

Ecology

The ecological success of a phytoplankton species is ultimately determined by its ability to optimise the balance between growth and loss processes. While the former is largely controlled by the availability and optimal utilisation of essential resources, such as light and nutrients, the latter includes factors such as cell sinking, cell mortality due to grazing, viral and parasite infection, as well as autolysis. Any investment successfully reducing mortality therefore weighs equally strong as cellular measures capable of increasing cell growth by the same magnitude. Coccolithophores apparently cover the entire range of the spectrum from growth-maximising to loss-minimising strategies. The ecological niche occupied by coccolithophores extends from subtropical, oligotrophic recycling systems to temperate and sub-polar, semi-eutrophic new production systems. Understanding the distribution and succession of coccolithophores in today's ocean requires detailed information on the physiological and ecological characteristics of the individual species. In this sense, *Emiliania huxleyi* is probably one of the best-studied phytoplankton species that is of relevance in the ocean. By comparing the physiological characteristics of coccolithophores with the environmental conditions under which they flourish, we will look for patterns explaining the seasonal and spatial distribution of *E. huxleyi* in relation to other dominant phytoplankton groups.

Due to vertical mixing of the upper surface layer, phytoplankton experiences high variability in light conditions. These stochastic changes are superimposed on diurnal and seasonal variation in solar irradiance. Seasonal changes in thermal stratification, which influence the depth of vertical mixing, further modify the light conditions encountered by a phytoplankton cell. Depending on their photosynthetic characteristics, phytoplankton differ in their ability to cope with variable

irradiance. While diatoms usually dominate in turbulent, low-stratified waters and are commonly the first to bloom when the water column begins to stabilize early in the seasonal cycle, *E. huxleyi* blooms predominantly occur in well-stratified waters in late spring/early summer, with mixed layer depths usually between 10 and 20 m, but always ≤ 30 m (Balch et al. 1991; Robertson et al. 1994; Tyrrell and Taylor 1996; Ziveri et al. 2000; Tyrrell and Merico this volume). This may indicate that the photosynthetic apparatus of diatoms is better adapted to operate efficiently under variable and, on average, lower light intensities. A comparison of photosynthetic parameters between the two groups, however, does not reveal any obvious characteristics which would imply a competitive advantage of diatoms over *E. huxleyi* under these conditions with the exception that *E. huxleyi* has a slightly higher light saturation irradiance for growth compared to diatoms (see above).

A distinct difference between diatoms and *E. huxleyi* is observed with respect to light inhibition. *E. huxleyi* has an unusual tolerance for high light intensity (Nielsen 1995; Nanninga and Tyrrell 1996). The lack of photoinhibition is surprising considering that photosynthetic carbon fixation of *E. huxleyi* is carbon-limited under ambient CO_2 concentrations (e.g. Paasche 1964; Nielsen 1995; Rost et al. 2003). The latter would imply that when suddenly exposed to high light conditions, *E. huxleyi* could potentially suffer from a shortage of electron acceptors. Elevated irradiances were found to stimulate calcification of *E. huxleyi*; especially under P-limitation (Paasche and Brubak 1994; Paasche 1998). It was therefore suggested that calcification provides a means to dissipate excess energy and thereby reduce the risk of photo-damage under high irradiances (Paasche 2002).

Surface layer stratification strongly reduces the input of nutrients from deeper layers. Highly stratified waters therefore are typically characterised by limited nutrient availability. *E. huxleyi* blooms generally coincide with relatively low levels of nutrients, in particular with respect to phosphate. Whereas nitrate concentrations during blooms of *E. huxleyi* are frequently at $4 \mu\text{mol L}^{-1}$ or higher, concentrations of ortho-phosphate are often lower than $0.2 \mu\text{mol L}^{-1}$ (Veldhuis et al. 1994; Fernandez et al. 1994; Van der Wal et al. 1995). Coccolithophore dynamics off Bermuda support this pattern, indicating increasing coccolithophore abundances to coincide with the seasonal advection of nitrate-rich but phosphate-poor waters to the euphotic zone (Haidar and Thierstein 2001). Coccolithophores may thrive best under such conditions due to their high affinity for nutrients, in particular for phosphate. In environments with fluctuating nutrient and/or energy supply diatoms are likely to dominate due to their higher capacity for luxury consumption compared to coccolithophores (Iglesias-Rodríguez et al. 2002; Falkowski et al. this volume).

Aside from physiological constraints regarding light and nutrient utilisation, grazer control equally influences the seasonal succession of the major phytoplankton groups. While the frustules of diatoms appear to serve as an effective mechanical protection against various types of microzooplankton predators (Hamm et al. 2003), the coccosphere of coccolithophores does not seem to provide the same degree of protection (Sikes and Wilbur 1982; Harris 1994). Due to its high intrinsic growth rate, microzooplankton has the potential to propagate at al-

most the same rate as its prey and thereby impede phytoplankton mass development. Grazing of copepods on microzooplankton can, in turn, considerably reduce the grazing pressure in this size class (cascade effect) and stimulate the microbial foodweb. Cascade effects of copepods on microzooplankton were in fact found to promote blooms of *E. huxleyi* (Nejstgaard et al. 1997). Due to the comparatively slow development of copepods and other carnivorous predators, mesozooplankton control typically occurs later in the seasonal cycle. This may be one of the reasons for the timing of *E. huxleyi* blooms, which are often confined to the late spring/summer period.

In summary, observed differences in photosynthesis versus irradiance relationships, nutrient uptake kinetics and food web interactions between *E. huxleyi* and other bloom-forming phytoplankton groups are - to a certain extent - in agreement with observed mass occurrences of *E. huxleyi*. For instance, *E. huxleyi* blooms coincide with relatively high nitrate and low phosphate levels and typically occur in stratified waters with high mixed layer irradiances. This is consistent with the observed tolerance of *E. huxleyi* to high light intensities, the low affinity for nitrate and high affinity for phosphate and organic phosphorus as well as a low capacity for luxury consumption. The relatively small cell size, which makes it more prone to microzooplankton grazing as compared to most diatoms, may repress mass developments of *E. huxleyi* at times and in areas where microzooplankton development is not kept in check by higher trophic levels. Despite the breadth of information recently becoming available on one of the best-studied marine phytoplankton species, our understanding of the complex interplay determining phytoplankton distribution and succession in the sea, however, is still insufficient to fully explain and forecast the geographical distribution and seasonal timing of *E. huxleyi* blooms.

Future scenarios

For about the last 20 million years atmospheric $p\text{CO}_2$ has remained ≤ 300 ppm (Berner 1990), fluctuating between 180 ppmv in glacial and 280 ppmv in interglacial times (Petit et al. 1999). With the beginning of the industrial revolution, burning of fossil fuel and changes in land use have disturbed this apparent balance. At present the $p\text{CO}_2$ has reached about 370 ppmv and is expected to rise to 750 ppmv by the end of this century (assuming IPCC's 'business as usual' scenario IS 92a). Since the atmosphere and ocean surface layer constantly exchange CO_2 , changes in atmospheric $p\text{CO}_2$ will invariably affect the surface ocean carbonate system. While increasing CO_2 causes the pH and CO_3^{2-} concentration to decrease, the large pool of HCO_3^- remains more or less constant. The expected increase in atmospheric $p\text{CO}_2$ is estimated to triple surface water CO_2 concentrations relative to pre-industrial values by the end of this century. Concomitantly, seawater pH and CO_3^{2-} concentrations will have dropped by 0.4 units and 50%, respectively (Fig. 2).

As a potent greenhouse gas, CO_2 has also been held responsible for the observed rise in average global temperatures. Global warming increases thermal stratification of the surface ocean, while enhanced freshwater input from melting ice lowers surface layer salinity. Both of these effects further increase density stratification of the upper ocean. Enhanced upper ocean stratification has two effects on phytoplankton: it reduces nutrient supply from deeper layers and increases light availability due to shoaling of the upper mixed layer (Fig. 3). How will these changes in marine environmental conditions affect coccolithophore growth and calcification and to what extent will it influence the distribution and productivity of *E. huxleyi* relative to other dominant phytoplankton groups (Fig. 8)? What will be the potential consequences for marine ecosystem regulation and ultimately, how does this affect the biological carbon pumps?

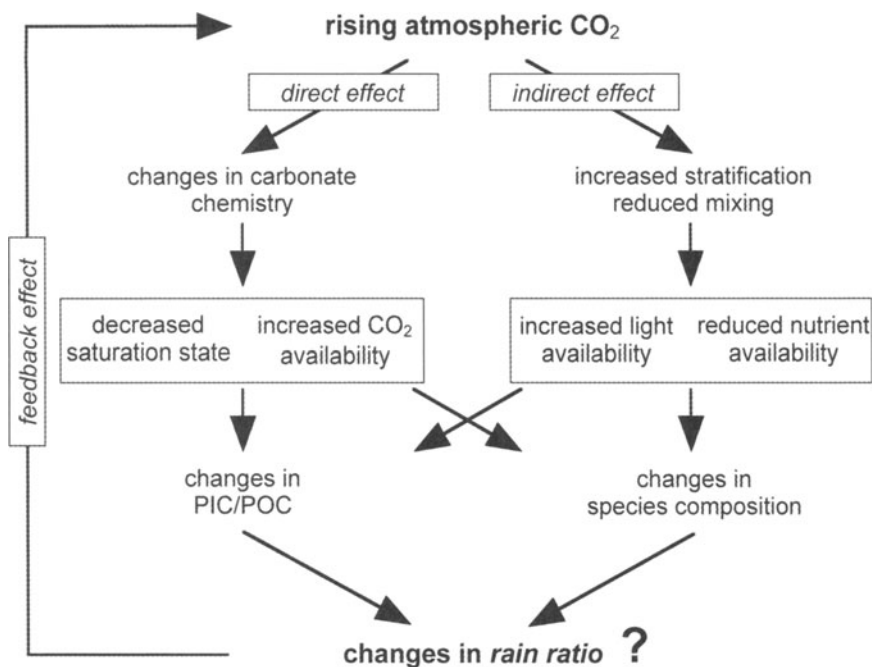


Fig. 8. Effects of rising atmospheric CO_2 on the surface ocean and its likely impact on phytoplankton: Increasing CO_2 levels directly cause changes in surface ocean carbonate chemistry, such as elevated CO_2 concentrations or decreased CaCO_3 saturation state. Indirect effects are those associated with enhanced stratification, such as increased light or reduced nutrient availability. These CO_2 related changes in the growth conditions are likely to affect the ecophysiology of coccolithophores and structure of phytoplankton communities, with possible consequences for the *rain ratio*.

Table 1. Predicted changes in abiotic factors (Houghton et al. 2001) and potential responses in photosynthesis, calcification, PIC/POC of coccolithophores as well as potential shift in the dominance between coccolithophores and diatoms (+, – and o denote an increase, decrease and no likely change, respectively). Changes in abiotic factors refer to the means of the upper mixed layer (UML). Potential responses in the physiology of coccolithophores were assessed on the basis of the expected changes in the UML and the sensitivity of respective processes. Potential changes in the dominance between coccolithophores and diatoms were assessed on the basis of the ecological niche of these two functional groups.

Changes in abiotic factors	Physiology			Ecology	
	Photosynth.	Calcific.	PIC/POC	Cocco./Diatoms	Biogeochem. Rain Ratio
increasing CO ₂ / decreasing pH	+ ¹⁻⁷	o ⁵ / – ^{4,7}	– ^{4,5,7}	+ ^{6/} – ⁴	+/- ?
increasing mean ir- radiance	+ ^{5,8-11}	+ ^{1,5,12-14}	+ ^{5,13,14} / – ¹⁵	+ ^{11,16}	+ ?
decreasing nutrient availability	– ^{7,17-19}	+ ^{7,17-19}	+ ^{7,17-19} / o ¹⁹	+ ¹⁹⁻²¹	+ ?

¹Paasche 1964; ²Nimer and Merrett 1993; ³Nielsen 1995; ⁴Riebesell et al. 2000; ⁵Zondervan et al. 2002; ⁶Rost et al. 2003; ⁷Berry et al. 2002; ⁸Paasche 1967; ⁹Van Bleijswijk et al. 1994; ¹⁰Nielsen 1997; ¹¹Nanninga and Tyrrell 1996; ¹²Van der Wal et al. 1995; ¹³Balch et al. 1996; ¹⁴Paasche 1999; ¹⁵Balch et al. 1992; ¹⁶Tyrrell and Taylor 1996; ¹⁷Paasche and Brubak 1994; ¹⁸Paasche 1998; ¹⁹Riegman et al. 2000; ²⁰Riegman et al. 1992; ²¹Egge and Aksnes 1992

In view of the inherent complexity of biological systems, including marine pelagic systems, it would be premature to attempt a forecast of marine phytoplankton responses to global change. In the following, we will outline possible, yet hypothetical scenarios for the effect of environmental change on coccolithophores (results of our assessment are summarized in Table. 1).

In principle, coccolithophores may benefit from the present increase in atmospheric pCO₂ and related changes in seawater carbonate chemistry. At pre-industrial CO₂ levels, rates of photosynthetic carbon fixation of *E. huxleyi* and *Gephyrocapsa oceanica* are well below CO₂ saturation. In comparison, photosynthesis of other bloom-forming phytoplankton groups such as diatoms and *Phaeocystis* are less CO₂-sensitive due to their efficient CCMs (Rost et al. 2003). Although under natural conditions CO₂ limitation is likely to be of minor importance for the proliferation of *E. huxleyi* compared to other limiting resources and loss processes, increasing CO₂ availability may improve the overall resource utilisation of *E. huxleyi* and possibly of other fast-growing coccolithophore species. If this provides an ecological advantage for coccolithophores, rising atmospheric CO₂ could potentially increase the contribution of calcifying phytoplankton to overall primary production.

On the cellular level the ratio of calcium carbonate to organic carbon production is expected to change as calcification and photosynthesis respond in different ways to CO₂-related changes in carbonate chemistry. Whereas the observed

stimulation of photosynthesis due to increased CO₂ availability increases the organic carbon quota of the cell, calcification remains constant or decreases in response to a decline in the calcium carbonate saturation state, depending on light conditions. As a consequence, the cellular PIC/POC ratio decreases with increasing CO₂. This effect has been observed under various irradiances and nutrient concentrations in lab experiments with mono-specific cultures (Riebesell et al. 2000a; Zondervan et al. 2002; Berry et al. 2002), as well as in *E. huxleyi*-dominated natural phytoplankton assemblages in a mesocosm study (Zondervan pers. com.). Whether this affects the ecological competitiveness of calcifying phytoplankton and to what extent it influences losses due to grazing, viral infection or autolysis, is presently unknown.

Whatever the purpose of calcification in coccolithophores, continued acidification of surface seawater due to rising atmospheric CO₂ will further deteriorate the chemical conditions for biogenic calcification. An overall decline in pelagic calcification is expected to decrease the ratio of calcium carbonate to organic carbon in the vertical flux of biogenic material (*rain ratio*), with corresponding effects on air/sea CO₂ exchange (see above). The overall effect will be an increase in the CO₂ storage capacity of the upper ocean, constituting a negative feedback to rising atmospheric CO₂ (Zondervan et al. 2001).

Changes in surface ocean stratification are likely to affect overall primary production as well as phytoplankton species composition. Enhanced stratification and the concomitant decrease in nutrient supply is likely to cause an overall decline in oceanic primary production as well as a poleward shift due to a longer growing season at high latitudes. Recent model calculations indicate large regional differences in the effects of climate change on the marine ecosystem, predicting a 20% decrease in export production in low latitudes and a 30% increase in high latitudes for a 2 × CO₂ scenario (Bopp et al. 2001). For coccolithophores the projected increase in stratification may prove advantageous since their blooms predominantly occur in well-stratified waters, often in subpolar latitudes. An increase in coccolithophore-dominated regions at the expense of diatom-dominated new-production systems would increase the global *rain ratio*. As high irradiances and low nutrient levels, but particularly the combination of both, have been shown to stimulate calcification relative to photosynthesis, enhanced stratification may also increase the PIC/POC ratio on the cellular level. This in turn may partly compensate for the reverse effect by CO₂-related changes in carbonate chemistry.

As illustrated above, a climate-induced increase in the contribution of coccolithophores to total primary production and a CO₂-related decrease in the cellular ratio of PIC/POC production, both of which are hypothetical at present, would have opposing effects on the marine carbon cycle. The net effect on carbon cycling ultimately depends on the relative importance and sensitivity of each of these processes to global change. In addition, possible changes at the level of primary producers, such as those outlined above, are likely to be amplified, compensated or reversed by interference from higher trophic levels, which themselves are bound to respond to environmental change. Understanding the complex structure and regulation of marine pelagic ecosystems, their responses to global environ-

mental change and the consequences thereof for marine biogeochemical cycling will be a major challenge for marine sciences in the coming decades.

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Dimethyl sulfide production: what is the contribution of the coccolithophores?

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Summary

It is hard to find a research paper or book on coccolithophores that does not include a few sentences on the role of this fascinating and enigmatic marine phytoplankton group in the production of dimethyl sulfide ((CH₃)₂S; DMS). Our aim here is to provide some general background information on DMS for non-specialists, but also to highlight current knowledge and what we believe to be significant gaps, for those with a specific interest in coccolithophores, other haptophytes and DMS.

Introduction

Lovelock et al. (1972) first discovered that DMS was ubiquitous in the sea and suggested that sea-to-air emission of this compound was a key pathway in the global sulfur cycle. Marine DMS emissions are thought to account for about 23% of the sulfur entering the global atmosphere but, because anthropogenic sulfur tends to deposit rapidly and close to source, the biogenic flux contributes 42% of the atmospheric column burden (Chin and Jacob 1996). Hence DMS inputs to the atmosphere are of greatest significance in areas that are distant from anthropogenic sulfur sources (Bates et al. 1987; Savoie and Prospero 1989; Twomey 1991). Once in the air DMS oxidizes quite rapidly mainly via hydroxyl radicals during the day, nitrate radicals at night (the latter being more significant in polluted areas), and reactions with halogen oxides may also be significant (Plane 1989; Ravishankara et al. 1997; Ballesteros et al. 2002). DMS oxidation leads to the formation of a number of sulfur compounds including sulfur dioxide, sulfate, methanesulfonic acid (CH₃SO₃H; MSA), dimethylsulfoxide ((CH₃)₂SO; DMSO) and dimethylsulfone ((CH₃)₂SO₂; DMSO₂).

In the 1980's and early 1990's research largely focused on the contribution of natural DMS emissions to the global sulfur budget, and the wet and dry deposition

of the acidic atmospheric oxidation products of DMS (natural 'acid rain'). However, there was a substantial shift in emphasis after Charlson et al. (1987) picked up on the earlier suggestion of Shaw (1983) and put forward the hypothesis that sulfate aerosols derived from marine DMS emissions influenced cloud albedo and global climate. A simple depiction is shown in Fig. 1. Sulfur aerosol influences climate in two ways: directly since aerosol particles scatter incoming radiation reflecting some of it back into space, and indirectly because the aerosols

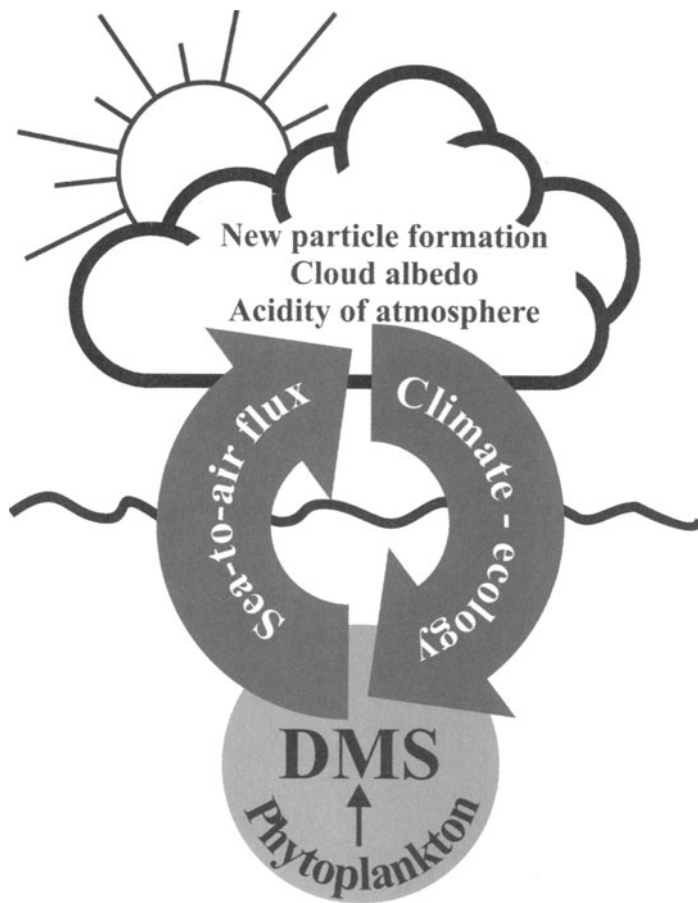


Fig. 1. Research on DMS has been stimulated by the "plankton-climate connection" hypothesis of Charlson et al. (1987). DMS derived from phytoplankton is emitted to the air and is the primary source of the sulfur particles that form in the atmosphere over the remote oceans. These acidic particles stimulate cloud formation and increase the Earth's reflectivity (albedo). In turn these processes affect climate and may also influence upper ocean ecology.

produced are of the ideal size and chemistry to act as cloud condensation nuclei, and these influence cloud albedo and cloud formation, again reflecting radiation from the sun and so cooling the earth. The so-called CLAW hypothesis (named after the initials of the authors Charlson et al. 1987) was controversial in that they suggested that the DMS-cloud albedo system would operate under negative feedback such that a climate change would be countered by an opposing change in DMS production. A great deal of debate ensued particularly regarding the climatic role of natural versus man-made aerosols derived from anthropogenic SO₂ emissions. Nonetheless, CLAW has provided great impetus for all aspects of research on DMS.

DMS is a biogenic trace gas derived from the cellular precursor dimethylsulfoniopropionate ($((\text{CH}_3)_2\text{S}^+\text{CH}_2\text{CH}_2\text{COO}^-)$; DMSP), a zwitterionic compound that is found in the cells of certain types of marine phytoplankton and seaweeds. In marine phytoplankton up to 20% of total cell carbon can be invested in DMSP (Matrai and Keller 1994; Matrai et al. 1995). As a result DMS and DMSP are essentially ubiquitous in the marine euphotic zone, although concentrations show considerable spatial and temporal variation. In recent years increasing attention has been paid to the biogeochemical, physiological and ecological roles played by DMSP and DMS. The haptophyte algae appear to be key players in global DMS production, and data obtained from blooms and cultures of *Phaeocystis* and the coccolithophore *Emiliania huxleyi* constitute a vital part of our current understanding of DMS/DMSP production, transformation and cycling.

DMSP – the major precursor of DMS

DMSP is considered a structural analogue of glycine betaine ($((\text{CH}_3)_3\text{N}^+\text{CH}_2\text{COO}^-)$) a quaternary amine compound that is well known as a compatible solute in many higher plants (Sakamoto and Murata 2002). Such organic compounds are accumulated so that plants can acclimate to environmental stresses such as changes in salinity and low temperature. DMSP has been shown to have some of the typical properties of a compatible solute (Kirst 1996). However, recent exciting research on trophic interactions (Wolfe et al. 1997; Steinke et al. 2002c) phytoplankton physiology (Stefels 2000) and the novel proposal that DMSP counters oxidative stress (Sunda et al. 2002), all suggest that DMSP functions way beyond its basic role as a compatible solute.

DMSP production by haptophytes

The seminal laboratory studies of Keller and co-workers give an excellent overview of cellular DMSP levels in 127 marine phytoplankton cultures covering representatives of 12 different algal classes (Keller 1989; Keller et al. 1989a, 1989b). The clonal cultures used were grown in nutrient rich media and analyzed

for total DMS plus DMSP content during the logarithmic phase of growth. From these studies a general 'rule of thumb' emerged that has been a guiding influence for many subsequent laboratory and field studies, i.e. that haptophytes and some dinoflagellates have higher intracellular levels of DMSP than diatoms. In Table 1 we review the Keller et al. data for haptophytes. The list of coccolithophores covers *E. huxleyi* and five other clones, all of which accumulated high intracellular concentrations of DMSP. Of the non-coccolith bearing haptophytes high intracellular DMSP levels were found in a range of species including *Chrysochromulina* spp., *Isochrysis galbana*, *Pavlova* spp., *Phaeocystis* spp. and *Prymnesium parvum*. Where data is available for several clones of one species considerable variation in DMSP levels can sometimes be observed. Whilst there is no doubt that the Keller et al. data is still a valuable resource, exceptions to the general rule mentioned above have emerged notably the high concentrations of DMSP in some ice diatoms (e.g. Kirst et al. 1991; Levasseur et al. 1994; Bouillon et al. 2002) and in planktonic diatoms under conditions of oxidative stress (Sunda et al. 2002). The considerable recent advances in the understanding of haptophyte phylogeny and ecology, and the increased availability of clonal cultures (see Van Lenning et al. this volume; Probert and Houdan this volume) would permit a far more comprehensive survey of DMSP in haptophytes today.

In addition to taxonomy, factors including low temperature, nitrogen and light availability may also influence the DMSP concentration observed in marine phytoplankton cells. Compatible solutes typically increase in concentration when the growth temperature is decreased and this is true for DMSP for some polar seaweeds (Karsten et al. 1992, 1996) and the prasinophyte *Tetraselmis subcordiformis* (Sheets and Rhodes 1996). Three pathways for DMSP synthesis have been elucidated (see Stefels 2000), but for a range of marine algae including *E. huxleyi* synthesis appears to be initiated by transamination of methionine. The pathway then proceeds via reduction and methylation reactions to a novel intermediate 4-dimethylsulfonio-2-hydroxybutyrate (DMSHB) and this is converted to DMSP via oxidative decarboxylation (Gage et al. 1997). The transamination step releases a valuable NH_3^+ group for general cell metabolism which fits with suggestions that DMSP would be preferentially synthesized under nitrogen limitation. In agreement with data in the early DMSP literature Keller and Korjeff-Bellows (1996) found that cellular DMSP increased with nitrogen limitation in a few marine phytoplankton, but a subsequent detailed investigation found no clear inverse relationship between cellular levels of glycine betaine and DMSP relative to nitrogen availability (Keller et al. 1999a, 1999b). Stefels (2000) suggested that DMSP synthesis could function as a metabolic overflow mechanism for excess reduced compounds and energy when growth is unbalanced e.g. due to nitrogen limitation. This would enable photosynthesis, methionine synthesis and other essential metabolic processes to continue. Cellular production of DMSP utilizes methionine, keeping its level low to prevent possible inhibitory feedbacks and releases N for essential metabolic processes.

Studies on DMSP and DMS production versus light intensity have produced rather mixed results. Vetter and Sharp (1993) found that *Skeletonema costatum*

Table 1. Summary of the Keller et al. (1989a, 1989b) and Keller (1989) data for total DMS plus DMSP for clonal coccolithophore and other haptophyte cultures expressed as equivalent cellular DMSP content and concentration data. The analysis technique used did not differentiate between extracellular and intracellular DMS and DMSP. Current clone designations and sites of isolation have been added (CCMP = Provasoli – Guillard National Center for Culture of Marine Phytoplankton <http://ccmp.bigelow.org/>). Note that the values for cultures of unidentified flagellates given in the 1989a paper have been omitted.

	Original clone name and CCMP code	Current clone name and CCMP code where changed or available	Site of isolation	pg DMSP per cell	μmol DMSP per cm ³ cell volume
COCCOLITHOPHORES	<i>Coccolithus neohelis</i> CONE	<i>Cruciplacolithus neohelis</i> CCMP298	Beach sand, Scripps, California Bight.	2.53	85.08
	<i>Emiliana huxleyi</i> BT6	CCMP373	Sargasso Sea	0.75	166.42
	<i>Emiliana huxleyi</i> 8613C	CCMP376	Gulf of Maine	1.10	124.4
	^a <i>Pleurochrysis carterae</i> COCCOII	CCMP645	Wood's Hole, Nantucket Sound, unknown	12.0	170.15
	^a <i>Syracosphaera elongata</i> SE62	^a <i>Pleurochrysis carterae</i> CCMP874		19.8	35.3
	<i>Umbilicosphaera sibogae</i> L1178	na	na	13.8	195.52
	unidentified 8613COCCO	na	na	1.1	125.37
OTHER HAPTOPHYTES	<i>Chrysochromulina ericina</i> NEPCC109A	CCMP281	North Pacific	3.81	251.49
	<i>Chrysochromulina herdlandis</i> NEPCC186	CCMP284	North Pacific	3.62	412.69
	<i>Chrysochromulina polylepis</i> ED1	CCMP1757	North Sea	6.14	191.79
	<i>Chrysochromulina polylepis</i> ED2	CCMP286	North Sea	11.7	394.78
	<i>Imantonia rotunda</i> IIE ₆	CCMP458	Gulf Stream	0.18	159.70
	<i>Imantonia rotunda</i> 1197NTA	CCMP456	Gulf of Mexico	0.26	87.31
	<i>Isochrysis galbana</i> CISO	CCMP463	Caribbean Sea	0.50	56.87
	<i>Pavlova lutheri</i> MONO	na	na	0.05	3.28
	<i>Pavlova pinguis</i> IG ₇	CCMP609	Sargasso Sea	0.71	46.87
	^b <i>Pavlova</i> sp. IIB ₃	CCMP617	Gulf Stream	0.22	53.36
	<i>Pavlova</i> sp. IIB ₃ ax	CCMP617	Gulf Stream	0.65	156.72
	^b <i>Pavlova</i> sp. IIG ₃	CCMP620	Gulf Stream	0.45	51.19
	<i>Pavlova</i> sp. IIG ₃ ax	CCMP620	Gulf Stream	0.52	59.18
	^b <i>Pavlova</i> sp. IIG ₆	na	na	0.65	73.66
	<i>Pavlova</i> sp. IIG ₆ ax	na	na	0.73	83.58
	<i>Phaeocystis</i> sp. 677-3	<i>Phaeocystis globosa</i> CCMP628	Caribbean Sea	2.29	260.45
	<i>Phaeocystis</i> sp. 1209	<i>Phaeocystis globosa</i> CCMP627	Gulf of Mexico	1.0	113.43
	<i>Prymnesium parvum</i> PRYM	CCMP708	Unknown	1.7	111.94

^a Name synonyms *Cricosphaera carterae* and *Hymenomonas carterae*

na = information not available

ax = axenic clones

^b Bacteria isolated from these clones showed no detectable DMS release.

produced 50% less DMS at $38 \mu\text{mol m}^{-2} \text{s}^{-1}$ than at $380 \mu\text{mol m}^{-2} \text{s}^{-1}$. The difference was more pronounced during senescence, but under low light the DMS production rate per cell matched or exceeded that of the high light culture during its extended log phase. In contrast the apparent release of DMS by *Phaeocystis antarctica* was minimal at $350 \mu\text{mol m}^{-1} \text{s}^{-1}$ and 26.6 fg DMS per pg cell carbon at $3.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, but there was no overall relationship between light intensity and DMS output (Baumann et al. 1994). Keller and Korjeff-Bellows (1996) found no significant or consistent common response to light intensity in terms of DMSP content in four microalgae including *E. huxleyi*. However, Arctic clones of *Phaeocystis* sp. were found to have higher rates of DMS production at low irradiance (Matrai et al. 1995) and Stefels et al. (1996) found that DMSP production continued in the dark period of a light/dark cycle in a temperate *Phaeocystis* clone. These data suggest that DMSP production is not directly linked to light and that the light history and overall cellular physiological state are the critical factors. Very few studies have considered the combined effects of nutrient and light stress. An excellent exception is the study of Stefels and Van Leeuwe (1998) who examined whether the overall effects of iron limitation in an Antarctic *Phaeocystis* clone, were due to reduced energy supply because of reduced photosynthetic activity or nitrogen insufficiency. They found that DMSP concentration increased under high light, iron-deficient conditions when *Phaeocystis* cells were close to nitrogen deficiency, but did not decrease under low light and iron limitation when the cells were more severely energy limited. Cell volumes were reduced significantly under low iron, but this reduction in volume was only accompanied by a reduction in DMSP content when light levels were low. At high light intensity cellular DMSP concentrations increased.

Recently, Sunda et al. (2002) put forward an exciting and intriguing hypothesis that DMSP, DMS, acrylate ($\text{CH}_2=\text{CHCOO}^-$), dimethylsulfoxide ($(\text{CH}_3)_2\text{SO}$; DMSO) and methane sulfinic acid ($\text{CH}_3\text{SO}_2\text{H}$; MSNA) and their related breakdown products constitute an antioxidant system in marine microalgae that may be regulated via DMSP lyase activity (DLA). All living cells have mechanisms to deal with reactive oxygen species (ROS) such as superoxide $\text{O}_2^{\bullet-}$, hydrogen peroxide H_2O_2 , singlet oxygen and the $\bullet\text{OH}$ radical. Oxidative stress occurs when a cell's capacity to prevent or repair ROS damage is exceeded. Environmental factors, e.g. osmotic stress, low temperature, nutrient limitation, high light, high or low temperature and chemical toxicity can increase ROS production levels. As already discussed some of these conditions are known to increase DMSP concentration in phytoplankton cells. In support of their hypothesis Sunda et al. (2002) demonstrated that in both *Thalassiosira pseudonana* (low constitutive DMSP) and *Emiliania huxleyi* (high DMSP) increased UV radiation, increased Cu^{2+} , CO_2 limitation and iron deficiency all increased cellular DMSP levels. However, it should be noted that another recent study found no increase in intracellular DMSP concentration when *E. huxleyi* was subjected to UV light (Van Rijssel and Buma 2002). Also the level to which marine phytoplankton depend upon this antioxidant system rather than well-established antioxidants such as glutathione or ascorbate is not yet known. In two separate field studies the 0.7–10 μm size fraction DMSP (Belviso et al. 1993) and DLA (Steinke et al. 2002b) were found to covary with

the concentration of photoprotective pigments. These data are complementary to the proposed hypothesis though no direct links between photoprotective pigment levels and oxidative stress were made.

Studies in coccolithophore blooms

From the field perspective, blooms of *E. huxleyi* are well known as areas of high DMS production and have provided an excellent model system for studying DMS production in a field situation. *E. huxleyi* is unusual amongst the coccolithophores in that it sheds its ornate calcium carbonate coccoliths, and given that these are small and numerous (approx. coccosphere diameter 5 μm , coccolith diameter 2.5 μm , coccolith weight 1.8 pg, 30 coccoliths per cell) they accumulate and amplify and scatter light, thereby affecting the optical properties of the surface ocean (Tyrrell et al. 1999). Such blooms can cover very large areas of the ocean and some stunning satellite images can be found in the literature and on various web sites (see Tyrrell and Merico this volume; Balch this volume). There has sometimes been a tendency to assume that the white water blooms seen via satellite are always dominated in terms of carbon biomass by *E. huxleyi*. However, in our experience of studies where detailed phytoplankton identification and enumeration has been carried out, this has been relatively rare. Indeed several field studies give strong indications that coccolithophores other than *E. huxleyi*, particularly *Calcidiscus leptoporus*, *Gephyrocapsa*, *Coccolithus pelagicus* and *Crystallolithus* (the motile holococcolith-bearing phase of *C. pelagicus*) may contribute significantly to the DMS levels observed in blooms of *E. huxleyi* (Turner et al. 1988; Holligan et al. 1987, 1993; Malin et al. 1993; Savidge and Williams 2001). In other *E. huxleyi* bloom studies nanoflagellates and/or dinoflagellates have contributed a significant portion of the particulate DMSP (DMSPp) observed (Archer et al. 2002b; Steinke et al. 2002a).

The ability to find and track white waters by accessing satellite images whilst onboard ship has been exploited during several DMS-focused field campaigns (e.g. Holligan et al. 1993; Malin et al. 1993; Matrai and Keller 1993). The most recent of these have been Lagrangian studies where the volatile tracer sulfur hexafluoride (SF_6) has been used to label and then track the movement and dispersal of an *E. huxleyi* population (Read and Pollard 2001; Burkill et al. 2002; Jickells et al. pers. com.). The principal use of the SF_6 tracer technique has been in studies designed to test the validity of parameterizations used to estimate air-sea fluxes of volatile compounds (Watson et al. 1991b; Nightingale et al. 2000), but following the suggestion of Watson et al. (1991a) the SF_6 mapping technique has been used to label open ocean areas with and without the simultaneous addition of nutrients. This powerful tool has led to significant advances in physical and biological oceanography e.g. during iron fertilization experiments (Martin et al. 1994; Boyd and Law 2001). Of particular note here are a 3.5 fold increase in DMS concentration following iron addition in the Pacific (Turner et al. 1996b), increases in prymnesiophyte DMSP followed by grazing-induced DMS production after iron

fertilization in the Southern Ocean (Boyd et al. 2000), a 4.7 fold increase in DMS over nine days in a northeast Atlantic cold-core eddy that most likely resulted from production associated with *C. pelagicus* (Savidge and Williams 2001), and studies which considered the production and turnover of DMSP and DMS in *E. huxleyi* blooms in the North East Atlantic and the northern North Sea which show the dominant influence of heterotrophic processes on the quantity of DMS available for sea-to-air exchange (e.g. Savidge and Williams 2001; Simó and Pedrós-Alió 1999; Archer et al. 2002b; Steinke et al. 2002a; Zubkov et al. 2002).

Studies in *Phaeocystis* blooms

Amongst the non-calcified haptophytes, *Phaeocystis* blooms have frequently been noted as hot spots for DMSP production and DMS emissions (see Liss et al. 1994). In late spring and early summer the phytoplankton population of the temperate southern North Sea is usually dominated by *Phaeocystis* and this influences water phase DMS concentrations and flux to the atmosphere in this region (Turner et al. 1996a; Van den Berg et al. 1996). There has also been considerable focus on Antarctic blooms of *Phaeocystis* (e.g. Gibson et al. 1990; Crocker et al. 1995; Turner et al. 1995; DiTullio 1996). Turner et al. (1995) compiled the published Antarctic data, derived a likely seasonal cycle for DMS and used this to estimate an annual emission of DMS to the atmosphere. There may be some bias towards coastal values in the data set, but the analysis indicated that Berresheim's (1987) earlier 86×10^9 mol value ($\sim 17\%$ of the marine biogenic flux estimate of Bates et al. 1992) was rather low. Hence the influence of the Southern Ocean on the sulfur cycle is probably disproportionately greater than its 5.6% of the global ocean area would suggest. On the basis of a study in the Barents Sea, Matrai and Vernet (1997) suggested that the role of the Arctic Ocean may be similarly underestimated. Studies on Arctic *Phaeocystis* clones (Matrai et al. 1995) are supportive of this underestimation. However, Bouillon et al. (2002) found high primary production but low DMS production in the polynyas of northern Baffin Bay, and the Leck and Persson (1996) estimate of 4 Gmol yr^{-1} for the total DMS flux from the northern high latitudes is less than 1% of the global estimate (Bates et al. 1992).

Overall the northern polar seas have received less attention and further studies are warranted to improve understanding of DMS emissions in this region.

Beyond phytoplankton blooms

A majority of studies on marine phytoplankton have focused on non-equilibrium, resource-driven situations such as spring and summer blooms in temperate and polar regions, and nutrient fertilization experiments. About 75% of all aquatic primary production occurs in the open oceans (Pauly and Christensen 1995), where lower chlorophyll levels remain fairly uniform over wide space scales for

long periods of time and picoplankton ($<0.2\text{--}2\text{ }\mu\text{m}$ diameter) are dominant in terms of primary production and biomass. Despite DMS emissions accounting for most of the non-sea-salt sulfate and MSA in the atmosphere over remote ocean areas (Savoie and Prospero 1989), and that it is in these regions distant from anthropogenic impact that DMS is likely to have the most influence on global climate (Charlson et al. 1987), these vast areas are relatively poorly studied in terms of DMSP and DMS production processes. Open ocean region DMS data mainly stem from studies carried out during research cruise transects which pass relatively quickly through different hydrographic zones. Such field campaigns are excellent for determining how DMS concentration varies over wide spatial scales and determining fluxes to the atmosphere. The database assembled by Kettle et al. (1999) is the most comprehensive compilation of DMS data. It contains 15,617 DMS values and has some coverage of open ocean areas. However, far smaller subsets of the data have associated DMSP or chlorophyll data, and the spatial coverage of these is poor, especially in the open ocean regions. It is clear that very few in-depth process type studies focusing on DMS and DMSP production have been done in the open ocean to date.

Turning now to the open ocean distribution of coccolithophores, coccolithophore diversity is highest in warm low-productivity waters (Winter and Siesser 1994), although the traditional view of coccolithophores as indicators of such regions has been challenged by recent studies in polar regions (Winter et al. 1999). At a very simplistic level the coccolithophore record in marine sediments gives an indication of species that were once present in the euphotic zone. The modern coccolith and carbonate flux sediment trap data (C. Sprengel, personal communication; Baumann et al. this volume) emphasizes strikingly two points that are relevant here. Firstly, that the cosmopolitan *E. huxleyi* is widely distributed and numerically dominant in sediments within and beyond the well-known white water areas. Secondly, other coccolithophore species are numerically important at stations in the Arctic and equatorial Atlantic, and much larger species (e.g. *Coccolithus pelagicus* and *Calcidiscus leptoporus*) dominate the carbonate export to the deep ocean throughout the Atlantic. Haidar and Thierstein (2001) examined living coccolithophore dynamics over 3 years near the BATS site in the oligotrophic North Atlantic gyre (Sargasso Sea). They found considerable seasonal and inter-annual variability. *E. huxleyi* dominated throughout the year in the upper 200 meters with maximum cell densities of $\sim 93,000\text{ cells l}^{-1}$. *Florisphaera profunda* showed cell densities up to $68,000\text{ cells l}^{-1}$ at 100–150 m depth. The highest densities of *Umbellosphaera tenuis*, 5000 cells l^{-1} were seen between 25–75 m, and *U. irregularis* occurred at a maximum concentration of 5000 cells l^{-1} in the upper 50 m. It is instructive to consider what such cell densities might mean in terms of field DMSP concentrations. *Umbilicosphaera sibogae* has $13.8\text{ pg DMSP per cell}$ (see Table 1), so if we guess at a $10\text{ pg DMSP per cell}$ for *U. irregularis* a cell density of 5000 cells l^{-1} might contribute 0.37 nmol l^{-1} to the total DMSPp concentration. The mean *E. huxleyi* concentration of $13,000\text{ cells l}^{-1}$ at $\sim 1\text{ pg per cell}$ (Table 1) would add an additional 0.1 nmol l^{-1} . The sum of these values lies towards the lower end of the range of DMSPp values reported by Dacey et al. (1998) for the same site, but at the very least this guesstimate underlines the idea

that the larger coccolithophores could make a significant contribution to the marine DMSP pool that can be equivalent to or larger than that of *E. huxleyi*.

Several studies in blooms and the open ocean have related DMS and DMSP concentration data to phytoplankton speciation and pigment. However, the phytoplankton speciation methods generally do not encompass the entire range of the coccolithophores. Many coccolithophores are known to have different life cycle stages (Billard 1994), but most studies have concentrated on the non-motile, diploid heterococcolith stage (made up of calcified elements of different sizes and shape) where the cell is covered with its highly characteristic placoliths. During the alternate haploid phase, the cells have crystalloliths or holococcoliths (having a single type of calcified element). Special efforts have to be made to accurately quantify and identify coccolithophores. Bollmann et al. (2002) review and compare the most common methods that have been used, and make recommendations regarding their application and limitations. Briefly, the Utermöhl (1958) settling method is in common use for routine quantification of phytoplankton assemblages, but generally a maximum volume of 100 ml is used and this is insufficient for species which occur at low concentration. Preservatives buffered at alkaline pH are essential to ensure that coccoliths do not dissolve and the method appears to consistently underestimate cell densities. Filtration methods are often preferred in studies that focus on coccolithophores and where only coccolithophores are counted, though uneven distribution of cells on the filters can be a major problem. However, much larger seawater samples e.g. 4–10 l can be used and the filters are desiccated and counted via polarizing microscopy. Where confirmation of identification is needed the same filters can also be examined using scanning electron microscopy. Some haptophytes, including *E. huxleyi* and *Phaeocystis*, also have a motile naked cell stage. Such small flagellates are at the limits of light microscopy and are very difficult to distinguish from other picoplankton. Analytical flow cytometry is often used for estimating numbers, but it is essentially impossible to identify and quantify this whole flagellate group to the species level with any accuracy. The taxonomic composition of the picoeukaryote flora of the open ocean has been addressed in recent studies using molecular techniques and novel lineages, including previously unknown haptophytes, have been identified (Moon-Van der Staay et al. 2000, 2001).

Detailed speciation and quantification data of the type described above has seldom been available during DMS-related field studies, and we are not aware of any studies that have included molecular analysis of phytoplankton speciation. Corn et al. (1996) found that a small range of picoplankton cultures had low but variable cellular DMSP levels with prymnesiophytes and prasinophytes showing the highest values. They applied an average DMSP per cell value to cell count data and obtained 0.7–2 μm fraction DMSP concentration data for a small set of oligotrophic Mediterranean samples. They concluded that the picoplankton groups contributed 10–35% of the DMSP in this small size fraction, but <1% of the total DMSP.

Quantification of specific pigments is being used increasingly to quantify the contribution of different taxonomic groups within mixed phytoplankton assemblages. The pigment 19'-hexanoyloxyfucoxanthin (HEX or HFx) is considered to

be diagnostic for haptophytes and has shown reasonable to good correlation with DMSP in several studies suggesting that haptophyte algae are key species for DMSP and DMS production. Belviso et al. (2001) examined pigment and DMSPp data for 200 samples from contrasting regions and concluded that the summed concentration of HEX and 19'-butanoyloxyfucoxanthin was a reasonable surrogate for DMSPp in the <10 μm nanoplankton fraction. However, using pigment data for quantitative purposes can be problematic given that pigment composition can shift in response to a cell's light history and physiological status. Furthermore, Van Lenning et al. (this volume) have found that some major haptophyte families do not synthesize HEX which suggests that single pigment comparisons may be insufficient.

To conclude this section, the combined evidence suggests that haptophytes, including the coccolithophores, are likely to be key species for DMS and DMSP production in the open ocean. However, very few direct in-depth process studies have been done, and the major evidence derives from simultaneous quantification of sulfur compounds and pigments, rather than the application of detailed identification and enumeration techniques. In the future the availability of suitable hierarchical molecular probes might allow us to better address the role of the uncultured haptophyte picoplankton in the flux of biogenic sulfur from the open ocean.

DMS release from DMSP

Although it is clear that DMSP is the key prerequisite for DMS production in marine waters, the pathway between cellular DMSP and a pool of DMS available for emission to the atmosphere is not straightforward. To summarize briefly, a complex web of processes is involved including phytoplankton growth, exudation, autolysis, grazer activity, viral lysis, bacterial consumption and transformation of DMSP, DMS and DMSO, the activity of algal DMSP lyase, photochemical production of DMSO and sedimentation. The total marine pool of DMSP is substantial, but only a very small proportion of it is ever emitted in the form of DMS to the atmosphere, the rest being recycled within the marine food web or sedimented to deeper waters. Many diagrams visualizing the biogeochemical cycle of DMS are available in the literature – excellent detailed examples can be found in the recent reviews by Kiene et al. (2000) and Simó (2001). For the purposes of the chapter we include a simple figure that summarizes current understanding of the main factors affecting the production and biogeochemical cycling of DMS. (Fig. 2).

Even though the amount of DMS emitted to the atmosphere is a small percentage of the total potential pool, the surface of the ocean is always supersaturated with DMS relative to its atmospheric concentration which ensures a continual flux to the air. The magnitude of the flux depends on the water phase DMS concentration and the transfer velocity, a parameter, which depends on wind speed, wave action, bubbles and surfactants. The physical sea-to-air gas exchange process will not be covered in any further detail here. The reader is referred to an excellent re-

cent book on gas transfer at water surfaces (Donelan et al. 2002) and previous publications that discuss estimating DMS fluxes to the air (Malin 1996; Turner et al. 1996a).

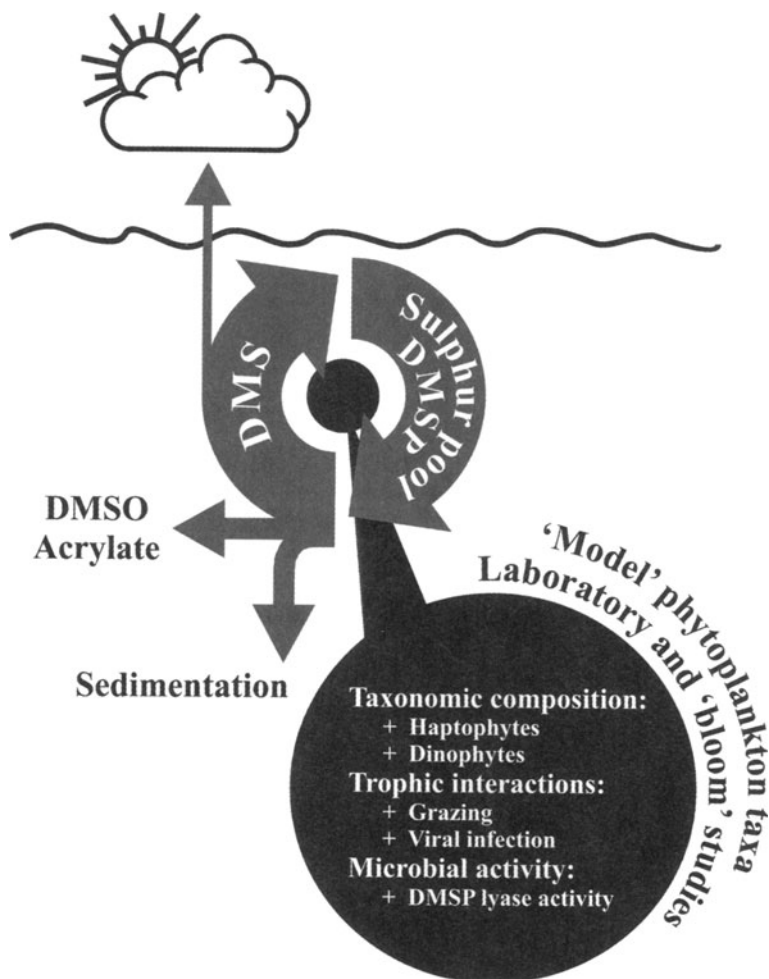


Fig. 2. Current understanding of the main factors affecting the production and biogeochemical cycling of DMS. Laboratory studies with selected plankton species and field studies in phytoplankton blooms indicate rapid cycling of DMS-sulfur in the microbial food web of the upper ocean. The DMSP-DMS production wheel is driven by taxonomic composition, trophic interactions and microbial activity. Some dinophytes and haptophytes (including the coccolithophores) are recognized as important producers of DMS. Grazing by zooplankton and viral lysis can further stimulate DMS production. Losses of DMS via sedimentation, and especially sea-to-air exchange, are considered to be small relative to the total DMSP plus DMS pool, and the roles of DMSO and acrylate are not well characterized.

Processes that influence the production of DMS from haptophyte DMSP

Phytoplankton cell lysis

Little DMS is produced in exponential phase cultures of DMSP-containing phytoplankton (e.g. Stefels and Van Boekel 1993). However, cells that are compromised in some way or lyse, release their DMSP and any intracellular DMS into the surrounding environment. Recent studies suggest that cell death or autolysis of marine phytoplankton cells may be more common than was previously assumed (e.g. Brussaard et al. 1995; Agusti and Duarte 2000), but its significance in DMS and DMSP release has not been quantified directly. The major pathways for the release of DMSP from phytoplankton cells appear to be grazing and viral lysis.

Grazing

Phytoplankton growth is always accompanied by grazing and it is not unusual for a large percentage of the marine phytoplankton to be grazed on a daily basis. Grazing can impact on the DMS cycle via incorporation of DMSP into biomass, by releasing DMSP to the water column during the feeding or digestion process and/or cleaving some or all of the DMSP to DMS prior to release. The quantity of cellular DMSP released and whether it is released as DMS or dissolved DMSP (DMSPd) has important consequences for DMS fluxes. Laboratory studies suggested that copepods and microzooplankton would influence DMS biogeochemistry (e.g. Dacey and Wakeham 1986; Wolfe and Steinke 1996), and further experimental evidence showed that microzooplankton-induced DMS production was related to the activity of the algal enzyme DMSP lyase (see below). However, until quite recently there was little firm evidence to demonstrate that grazing impacts on DMS production in the field.

The method most commonly used for estimating microzooplankton grazing rates is the dilution technique (Landry and Hassett 1982; Landry et al. 1995). This method was adapted by Archer et al. (2001, 2003) who combined the usual measurements of net phytoplankton growth rate, with analysis of DMSPp, DMSPd and DMS concentration, and bacterial abundance and net growth rate. In addition bacterial consumption of DMS was estimated in parallel incubations using the inhibitor dimethyl disulfide (Wolfe and Kiene 1993). In three experiments with temperate seawater it was estimated that 14, 15 and 9% of the ambient DMSPp was converted to DMS+DMSPd by grazing (Archer et al. 2001). In a further study, estimates of the *Phaeocystis globosa* DMSPp ingested by microzooplankton accounted for the DMS production rates observed (Archer et al. 2003). It was noted that extreme care had to be taken so that the filtration steps needed for setting up the dilution series did not perturb DMS and DMSPd levels. In a parallel

study Wolfe et al. (2000) concluded that rapid microbial consumption and artificial release of DMS and DMSP during filtration compromised this approach for samples from the Labrador Sea. However, a multidisciplinary Lagrangian study in a developing phytoplankton bloom in the northern North Sea met with more positive results. High DMSPp production rates and similar DMSPp loss rates were observed suggesting high turnover. Microzooplankton grazing accounted for an average of 91% of the DMSPp lost and most of the ingested DMSPp was released as DMSPd rather than DMS (Archer et al. 2002b). The evidence to date suggests that microzooplankton grazing is a major route for the release of DMSPp to seawater, but the modification of the dilution technique does not separate changes in DMS due to grazing from DMS production and loss due e.g. to bacterial activity and viral lysis. A selective technique for direct quantification of grazing-mediated DMS production would allow a more straightforward approach to understanding what controls DMS concentration in the sea.

Viral lysis

High concentrations of viruses are ubiquitous in seawater and a significant fraction of these infect marine phytoplankton including *E. huxleyi* and *Phaeocystis pouchetii*. Malin et al. (1992, 1994) first suggested that viral lysis of marine phytoplankton cells could be an important route for the release of DMSP and DMS to seawater. Subsequently, viruses specific to culturable DMSP-containing hosts became available (Jacobsen et al. 1996), and allowed us to test this hypothesis in the laboratory. Infection of a Norwegian strain of *P. pouchetii* led to four fold increase in DMS concentrations over control culture levels 20 hours after virus addition, with an increase to eight fold after 45 hours (Malin et al. 1998). In a parallel study Hill et al. (1998) observed DMS production when a non-axenic culture of *Micromonas pusilla* lysed following viral infection, but no DMS release when axenic cultures were used. The simplest explanation for this would be that *M. pusilla* has no DLA, but nothing is yet known of DLA in this strain. Molecular analysis of *E. huxleyi*-specific viruses (*EhV*) isolated from a Norwegian fjord mesocosm and the Plymouth, U.K. coast shows that they have large double stranded DNA genomes ~410 kb in size. Phylogenetic analysis based on their DNA polymerase genes places *EhV* in the new Coccolithovirus genus within the *Phycodnaviridae* family of algal viruses (Schroeder et al. 2002; Wilson et al. 2002b). Current work in our laboratory is examining viral lysis and DMS production using the *E. huxleyi*-*EhV* couple with the aim of defining any link between DMS production and DLA (Evans, pers. com.). However, all the virus isolates available so far infect only low-lyase activity strains of *E. huxleyi*.

With currently available techniques, quantifying the relative contribution to DMS production of viral lysis versus grazing in the natural environment remains a tough challenge. However, a promising modified dilution protocol for estimating viral mortality of phytoplankton has recently been developed (Evans et al. 2003). In an *E. huxleyi* mesocosm bloom study in 2000 we observed maximum DMS concentrations after a substantial increase in the numbers of the large viruses that

typically infect phytoplankton cells (quantified by analytical flow cytometry) and a concomitant collapse in the *E. huxleyi* population, which suggested a link between viral lysis and DMS production (Darroch et al. pers. com.). However, in an open seawater *E. huxleyi* bloom Lagrangian study in the northern North Sea, Wilson et al. (2002a) found no link between the numbers of large viruses and DMS or DMSP production, and it was concluded that microzooplankton grazing out-competed viral infection. Further research is necessary to determine how viruses impact on the biogeochemical DMS cycle in the natural environment.

DMSP lyase activity

The major pathway for the production of DMS from DMSP is thought to be via the action of an enzyme known as DMSP lyase (dimethylpropiothetin dethiomethylase, enzyme classification number 4.4.1.3) which cleaves DMSP to yield DMS, acrylate ($\text{CH}_2=\text{CHCOO}^-$) and a proton. Several isozymes have been characterized and enzyme activity is strain specific and spans a wide range. DLA has been found in some marine phytoplankton, a marine fungus, a heterotrophic dinoflagellate and some seaweeds (see Steinke et al. 1996). However, despite DMSP lyase being a key enzyme in the biogeochemical cycle of DMS, detailed information is rather limited, and the relative importance of bacterial and phytoplankton lyase enzymes is unclear. Amongst the haptophytes most attention has been paid to clones of *E. huxleyi* and *Phaeocystis*. In a *Phaeocystis globosa* isolate from the North Sea the rate of production of DMS from DMSP added directly to cultures was highest in young exponentially growing cultures and declined with culture age (Stefels and Van Boekel 1993). Further studies using crude extracts revealed the extracellular location of the enzyme and its association with the membrane fraction (Stefels and Dijkhuizen 1996), and in the field *in vitro* assays for DLA data correlate strongly with *Phaeocystis* numbers (Stefels et al. 1995). In *E. huxleyi* DMSP lyase is located inside the cell and seems to be separated from DMSP, such that DMS production occurs when cellular integrity is compromised, e.g. when grazed by microzooplankton (Wolfe and Steinke 1996; Steinke et al. 1998) or infected and lysed by viruses (Malin et al. 1998). Wolfe et al. (1997) demonstrated that, when offered a choice of prey, the heterotrophic dinoflagellate *Oxyrrhis marina* avoided grazing on strains of *E. huxleyi* with high constitutive levels of DLA. Acrylate has antibacterial properties at high concentration, so it was proposed that *E. huxleyi* uses DMSP as the basis of a grazing-activated defense mechanism, whereby the acrylate produced during grazing deters microzooplankton from eating high-lyase strains when other food sources are available. It is also thought that the volatile product DMS could function as a signaling compound in trophic interactions. A full discussion of the role of climate relevant volatiles and related compounds in trophic interactions can be found in a review by Steinke et al. (2002c).

Two field studies in *E. huxleyi* blooms have investigated *in vitro* DLA. In the North Atlantic (Steinke et al. 2002a) activity was associated with particles $>10\ \mu\text{m}$ and correlated with the dinoflagellate pigment peridinin. In the northern North Sea

profiles of DLA, dimethylsulfoxide (DMSO) and photoprotective pigments were coincident (Steinke et al. 2002b). It was suggested that DMSP lyase enzymes could be affected by light and that the data was supportive of the recent Sunda et al. (2002) hypothesis which suggests that DMSP and its cleavage products are involved in scavenging reactive oxygen species.

Little is known of DLA in other haptophyte phytoplankton, but interestingly preliminary data from a recent study suggest that neither *Gephyrocapsa oceanica* (National Institute for Environmental Studies, Japan clone NIES-353) nor *Isochrysis galbana* (CCMP 1324) have any significant intracellular or extracellular DLA (Niki et al. 2000).

Bacterial DMSP lyase enzymes present a problem when quantifying algal DLA in mixed assemblages. A field study by Scarratt et al. (2000) approached the question of the contribution of free and particle attached or associated bacteria to DLA by analyzing filter fractionated seawater samples. They found that DLA was associated with the larger phytoplankton-containing size fractions, and particle associated bacteria had apparent half-saturation constants for DMSP and DMS that were ~10 times that of the free-living fraction. Assuming that DMS and DMSP concentrations are enriched in the phycosphere suggests that different bacterial communities have the ability to utilize these compounds at a level that reflects the concentration in their immediate microenvironment.

Overall understanding of the biochemistry, physiology and ecology of DMSP lyase is incomplete and requires further research. In particular a simple and reliable assay for phytoplankton *in vivo* DLA is needed to enable more realistic quantification of DMS production via this pathway in natural assemblages where intracellular and extracellular membrane-associated enzymes may be present. Steinke et al. (2000) published a small data set for such a technique which shows some potential. For clonal axenic cultures of *E. huxleyi* CCMP373 and 379 they found that *in vivo* rates of DLA were 2.8–5 times lower than *in vitro* values, and differences of a similar order were found with three samples from the North Sea (1.7, 2.6 and 2.9 times lower). Possible problems that were noted included handling and concentrating the phytoplankton biomass so as to cause minimal cell perturbation, the choice of buffer pH and temperature for the assay and sample storage. A good database for *in vivo* DLA would enable a much better appraisal of the ecophysiological role of this important algal enzyme.

Other key compounds in the DMS biogeochemical cycle

Acrylic acid

Despite the possible roles of acrylate ($\text{CH}_2=\text{CHCOO}^-$; the form of acrylic acid dominant at seawater pH) as a grazing deterrent and intracellular antioxidant, few studies have focused directly on this compound. In Antarctic coastal waters during blooms $0.001\text{--}1.21\ \mu\text{mol l}^{-1}$ concentrations have been found (Yang et al. 1992, 1994; Gibson et al. 1996), but the major barrier to more in-depth studies has been

the difficulty associated with the analysis of this highly soluble compound at the nanomolar concentrations that are likely in less productive waters. However, a direct seawater injection HPLC technique developed in our laboratory is showing considerable promise and should allow us to learn much more about the role of this 'forgotten' compound in the biogeochemical DMS cycle (Kadner, pers. com.). There has been a tendency in DMS related research to 'forget' that acrylate may derive from other potential metabolic routes and not solely and directly from DMSP cleavage. These are:

- Demethiolation of the product of DMSP demethylation 3-methiolpropionate (MMPA; $\text{CH}_3\text{S}^+\text{CH}_2\text{CH}_2\text{COO}^-$) which yields acrylate and methanethiol (CH_3SH).
- Breakdown of 3-mercaptopropionate (MPA; $\text{HS}^+\text{CH}_2\text{CH}_2\text{COO}^-$) in a reaction which might also yield H_2S (see Taylor 1993; Kiene et al. 2000).
- Via reaction of the quaternary ammonium analogue of DMSP alanine betaine ($\text{CH}_3\text{N}^+\text{CH}_2\text{CH}_2\text{COO}^-$) with OH^- (King 1988).

In an Adriatic Sea study Slezak et al. (1994) investigated whether acrylic acid might influence bacterial incorporation of leucine and thymidine. They found some growth inhibition in 20 minute incubation experiments with acrylic acid concentrations of 1 mmol l^{-1} or more, and in 24 to 110 h experiments activity was reduced when the concentration was $>10 \text{ } \mu\text{mol l}^{-1}$. They concluded that the growth of bacteria would rarely be inhibited by acrylic acid at concentrations equivalent to the nanomolar seawater concentrations of DMS, but suggested that its role in cell aggregates should be investigated. In a study on *Phaeocystis* cultures Noordkamp et al. (1998) found the concentration of acrylate in the medium increased from $0.1\text{--}1.0 \text{ } \mu\text{M}$ during exponential growth to $1\text{--}4 \text{ } \mu\text{M}$ in stationary phase. Most of the acrylate was located in the colony mucus and they suggested that microscale concentrations up to $\sim 1\text{--}6 \text{ mM}$ could exist. Although such acrylate levels could have antibacterial activity, healthy *Phaeocystis* colonies are enclosed by a strong, semi-permeable skin, which is thought to act as a physical barrier to bacteria, grazers and viruses (Hamm et al. 1999). Nevertheless, when colonies begin to decay the acrylate is likely to be readily released and therefore available for consumption by the acrylate-utilizing bacteria that are common in *Phaeocystis* blooms (Noordkamp et al. 2000).

DMSO

The current evidence suggests that dimethyl sulfoxide (DMSO) in seawater derives from abiotic photooxidation of DMS (Brimblecombe and Shooter 1986; Shooter and Brimblecombe 1989; Kieber et al. 1996; Brugger et al. 1998; Hatton 2002) and the release of particulate DMSO from phytoplankton cells (Simó et al. 1998; Lee and de Mora 1999a; Simó et al. 2000; Lee et al. 2001). Bacterial oxidation of DMS is also possible, but this has yet to be proven for natural seawater samples. Wet deposition of DMSO formed by atmospheric oxidation of DMS may provide an additional source to the water column. The principle loss mechanisms

for marine DMSO could be via abiotic or biotic oxidation to downstream products (e.g. dimethyl sulfone) or bacterial transformation/utilization. DMSO has been the subject of increasing attention in recent years due to the development of more sensitive and selective analytical methods (reviewed by Simó 1998), but despite its ubiquity in marine waters, DMSO is still a rather poorly understood component of the marine sulfur cycle (reviewed by Lee and de Mora 1999b). It is recognized that DMS oxidation and DMSO reduction may be important in controlling sea surface concentrations of DMS and hence, the quantity of DMS available for sea-to-air gas exchange. Nevertheless, the major outstanding questions are whether, and/or under what circumstances, DMSO acts as a source or a sink for marine DMS.

Bacterial activity influences the size of the DMS pool in seawater

Marine phytoplankton invest up to 15–20% of cell carbon in DMSP (Matrai and Keller 1994; Matrai et al. 1995) and DMSP can account for 50–100% of the particulate sulfur in some species (Matrai and Keller 1994). The total amount of carbon and sulfur sequestered in DMSP and DMS means that these compounds influence the global carbon cycle as well as the sulfur cycle. It is also worth emphasizing that every molecule of DMS emitted to the atmosphere takes along two carbon atoms for each sulfur atom. DMSP supports 1–13% of the bacterial carbon demand in surface seawater, they can also retain it for use as an osmolyte, and it also appears that DMSP can be a major sulfur source for marine bacteria being more energetically favorable than sulfate (Kiene et al. 2000).

Given the focus of this chapter it is inappropriate to review the large body of literature on the utilization of DMS and related compounds by cultures of aerobic and anaerobic bacteria, natural bacterioplankton assemblages and sediment slurries (see reviews by Taylor 1993; Taylor and Visscher 1996). Rather we will concentrate on a few recent studies that have advanced this area significantly. Kiene and Linn (2000) pioneered the use of tracer quantities of ^{35}S -DMS and ^{35}S -DMSP to quantify the turnover and fate of these compounds in seawater. They found that in coastal waters about 40% of the DMSP sulfur is rapidly incorporated into particulates and a further 40% into a dissolved non-volatile fraction (including sulfate), whereas in oceanic waters the values were 6–25% and 68–75% respectively. At lower ambient temperatures and higher DMSP concentrations volatile products dominated over particulate products, suggesting that bacterial sulfur demand was saturated. In all cases the volatile fraction was dominated by methanethiol (CH_3SH) and was consumed within 1–3 hours, whereas DMS was the minor product and turned over more slowly. Methanethiol readily complexes with metals and dissolved organic matter which retards its flux to the air. Radiotracer experiments show that the methiol group of methanethiol is efficiently incorporated into methionine, suggesting that it is a key intermediate in the pathway leading to incorporation of DMSP-sulfur into bacterial protein (Kiene et al. 1999).

DMSP and DMS are valuable substrates for marine heterotrophic bacteria, and bacterial uptake, consumption or transformation processes limit the emission of climatically active DMS to the atmosphere. The DMSP demethylation/demethiolation pathway appears to be the dominant sink for DMSP and DMS is usually a minor product. On the basis of culture and seawater studies Kiene et al. (2000) put forward the hypothesis that DMSP 'availability' controls DMS production, whereby as DMSP concentration increases the fraction of the DMSP pool converted to DMS increases until the demethylation pathway is saturated and at this point the amount of DMS produced begins to increase. This hypothesis does not necessarily require high absolute concentrations of DMSPd, since the key is the level of bacterial sulfur demand relative to DMSP availability. It is also important to note that bacterioplankton growth in the sea can be limited by nutrient availability, UV light, bacteriophage and grazing. Indeed, Simó and Pedrós-Alió (1999) proposed a relationship between mixed-layer depth (MLD) and the DMS yield from DMSP, whereby UV-B radiation reduces bacterial activity when the MLD is shallow. Additionally, studies by Zubkov et al. (2001, 2002) and Gonzalez et al. (1999) highlight that bacterial speciation is also likely to be important since common species related to the genus *Roseobacter* and other alpha-proteobacteria seem to be highly active in DMSPd consumption.

Modeling DMS biogeochemistry

From the discussion so far it is clear that in the past 10 years our knowledge of the processes that affect DMS production from phytoplankton DMSP has increased very significantly, and it seems likely that most of the key processes have been identified. However, it remains hard to determine experimentally which processes dominate in natural marine waters for two main reasons. Firstly techniques used for quantifying the different processes are not necessarily compatible because of different experimental time-scales, dark incubation rather than natural light, or seawater screened for larger zooplankton or left whole. Secondly, even with the range of techniques currently available, a comprehensive field study needs a large research ship and scientific crew. Such studies are logistically demanding and extremely expensive to mount. Nevertheless very good data sets and insight into DMS production can be gained. A series of DMS biogeochemistry papers published in Deep-Sea Research Part II in 2002 are an excellent example of such a study where a developing coccolithophore bloom was labeled with SF₆ and followed for six days (see overview by Burkill et al. 2002). Over this period micro-zooplankton grazing converted most of the DMSPp to DMSPd with little DMS production, bacterial consumption dominated DMSPd turnover with 16% at most converted to DMS, bacteria also removed 62–98% of the DMS, the flux of sulfur to the air accounted for 10% of the water column DMS production and 1.3% of the DMSPp production, and the total DMS turnover time was estimated at 0.4–1.6 days (Archer et al. 2002a). To further explore DMS/P production in this *E. huxleyi* bloom, the authors went on to incorporate their DMS biogeochemistry data into an

existing 1-D ecosystem model and use sensitivity analysis to investigate the relative importance of various processes. This underlined the importance of grazing as a mechanism for the release of dissolved organic matter, and bacterioplankton production in determining the yield of DMS from DMSP. Archer et al. (2002a) concluded that the DMS yield (i.e. the point at which DMS is produced in the pathway from DMSPp to DMS) can be variable even within the progression of a single bloom. The yield being greatest when DMS is produced directly via phytoplankton cell lysis or when cells are grazed, and lower DMS production occurs when the bacterioplankton demand for DMSP-sulfur is high.

There have been many other attempts to model and predict DMS emissions to the atmosphere. This facet of the DMS research area is moving very rapidly and we look forward to further developments. We will consider just a few key studies here. Kettle et al. (1999) and Kettle and Andreae (2000) compiled over 15,000 DMS data points to construct global DMS maps and interpolated these with respect to other published data and knowledge of biogeochemical provinces. Such databases are a fantastic resource for DMS modeling research, though utility can be limited by the inherent under-sampling of some areas and during the autumn and winter seasons. Anderson et al. (2001) extended the Kettle database by merging it with additional data and developed a 'broken-stick' regression equation which predicts DMS from gridded chlorophyll, light and nutrient values. The patterns of DMS distribution derived from this were consistent with observations for high latitude, upwelling and shelf areas, but did not work so well for low-DMS concentration regions.

Gabric et al. (2001) extended their previous modeling analysis of DMS production in the Subantarctic Southern ocean where biogenic sulfur dominates (Gabric et al. 1998) with the aim of simulating and evaluating the DMS-climate link. They used a full ocean-atmosphere coupled general circulation model to force a DMS production model, and the results suggest a 1–6% increase in DMS flux between 1960 and 2080. An increased flux of this size would result in a -0.3 W m^{-2} radiative forcing alongside a tripled- CO_2 forcing of $+6.9 \text{ W m}^{-2}$, i.e. a negative feed-back, though the analysis assumes no changes in marine food web structure over the period. Taking a somewhat similar approach, and using data obtained during several cruises, Bopp et al. (2003) derived a global DMS distribution similar to contemporary observations for a $1 \times \text{CO}_2$ scenario, and at $2 \times \text{CO}_2$ the model predicted a +2% average change, though spatial heterogeneity was large (-15 to $+30\%$). Aumont et al. (2002) present a model of the global distribution of DMS concentration which uses relationships formulated with Atlantic data sets applied to an update of the Kettle data set. This model was added into an existing global ocean carbon cycle model and predicted DMS and DMSPp concentration fields compared reasonably well with observations, apart from an underestimation of seasonal variability in the high latitudes. Interestingly the Aumont model indicates that $\sim 30\%$ of the global DMS flux occurs in the subtropical/subpolar frontal zone of the Southern Ocean, and that the area south of the Polar Front is a modest source of DMS.

Finally building on initial results which suggested a relationship between vertical mixing and DMS production (Simó and Pedrós-Alió 1999) such that DMS

concentration is highest when the mixed layer depth is shallow, Simó and Dachs (2002) constructed an algorithm for predicting DMS concentration from satellite chlorophyll and climatological physical data. The inverse relationship they find between DMS concentration and mixed layer depth appears to hold for ~80% of the global ocean. Previous attempts to model DMS production from chlorophyll have been less successful because of the taxonomic variability in DMSP production and the critical role of grazers, viruses and bacterial activity in DMS production. Simó and Dachs (2002) assert that their simplistic approach works because the combination of chlorophyll and mixed layer depth shifts the control on DMS production away from phytoplankton to the interaction between the microbial food web and the physical environment. Hence, it side-steps the need for accurate knowledge of all the DMS production processes and their interaction.

Significance of DMS production by coccolithophores

It is clear that at the present time we have very scant knowledge about the DMS and DMSP production potential of coccolithophores other than the cosmopolitan *E. huxleyi*. Hence, in attempting to assess the significance of DMS production by this group we must focus initially on sulfur emission rates derived for *E. huxleyi* blooms. A range of DMS flux values, calculated using the Liss and Merlivat (1986) sea-to-air gas transfer parameterization, are shown in Table 2. It should also be noted that the errors associated with calculating DMS flux rates are large and that this compilation is not a comprehensive list of reported values, though it does cover a number of different bloom and non-bloom areas in various different regions. DMS emission rates in excess of $15 \mu\text{mol m}^{-2} \text{d}^{-1}$ have been reported for *E. huxleyi* and *Phaeocystis* blooms. High DMS flux rates most often occur when high winds follow a period of calm weather and high DMS concentrations in the water column. For example the value of $44.4 \mu\text{mol m}^{-2} \text{d}^{-1}$ reported for the *E. huxleyi* bloom studied during the ACSOE Lagrangian study corresponded with wind-speeds in excess of 25 km h^{-1} . Somewhat lower sulfur emission rate values of up to $7 \mu\text{mol m}^{-2} \text{d}^{-1}$ have been derived for *E. huxleyi* blooms in the Gulf of Maine and northern North Sea. These lower emission rates are more in line with the range of estimates reported for oligotrophic and mesotrophic open ocean areas. However, high 'spot' sulfur emission values have also been derived from data for the Pacific, Indian, Southern and Arctic Oceans, and the South China Sea. So high DMS emission rates are not exclusive to haptophyte blooms.

To put these values into some sort of global context we now consider the total input of biogenic sulfur to the atmosphere. There are many estimated values in the literature, but the value of $0.5 (+/-0.33) \text{ Tmol a}^{-1}$ for the marine biogenic flux from Bates et al. (1992) takes into account seasonal and regional variations in DMS production. Taking the value of $3.62 \times 10^8 \text{ km}^2$ for the area of the global ocean (Couper 1989), this biogenic flux of sulfur would require a daily DMS emission rate of $3.78 (+/-2.5) \mu\text{mol m}^{-2} \text{d}^{-1}$ over the whole ocean surface. This

Table 2. A selection of sulfur emission rates from the literature. For comparability only values estimated using the Liss and Merlivat (1986) wind speed based sea-to-air gas exchange parameterisation are given. It should be noted that up to ~30% higher DMS flux values would be obtained if the more recent parameterisations proposed by Wanninkhof (1992), Wanninkhof and McGillis (1999) or Nightingale et al (2000) were used. The whole ocean ($3.62 \times 10^8 \text{ km}^2$) value given is the average DMS flux needed to fulfil the estimated total marine biogenic flux of $0.5 (+/- 0.33) \text{ Tmol a}^{-1}$ (Bates et al. 1992)

Area	DMS flux $\mu\text{mol m}^{-2} \text{ d}^{-1}$ mean and/or range	Reference
Whole ocean	3.78 (+/- 2.5)	see legend
<i>Emiliania huxleyi</i> blooms		
North East Atlantic June–July 1991	26.9	Holligan et al. 1993
North East Atlantic June–July 1987	^a 20.7	Malin et al. 1993
Gulf of Maine July 1990	0.94 – 6.25	Matrai and Keller 1993
^b Northern North Sea June–July 2002	6.0	Archer et al. 2002a
^b North East Atlantic June–July 1998	^c 0.24 – 44.4	Jickells et al. personal communication
<i>Phaeocystis</i> blooms		
GB Shelf July–August 1985	29.2	Turner et al. 1988
Faeroe Islands area June–July 1987	11.4	Malin et al. 1993
Southern North Sea June 1989	16.4	Turner et al. 1996a
Southern North Sea summer 1989	5.92	Turner et al. 1996a
Other areas		
North Pacific 1982–1985	4.97 (2.11 – 11.84)	Bates et al. 1987
Eastern tropical Pacific February 1989	13.6 (0.5 – 36)	Kiene and Bates 1990
North East Pacific April 1991	0.8	Bates et al. 1994
Central Arctic August to October 1991	0.6 (~0 – 19)	Leck and Persson 1996
South China Sea Nov–Dec 1993	7.6 (0.19 – 20.6)	Yang et al. 1999
^d Southern Ocean Spring–Summer 1991–1995	9.4 (1.7 – 49)	Curran and Jones 2000
Indian Ocean Jan–March 1998	1.0 (0.1 – 3.1)	Shenoy et al. 2002
Indian Ocean Jan–March 1999	7.2 (0.2 – 29.1)	Shenoy et al. 2002

^afor area west of 10°W with high coccolithophore abundance.

^bLagrangian studies using sulfur hexafluoride as a tracer.

^cRange of mean values for nine patch surveys. The *E. huxleyi* bloom was within the cold core of an anticyclonic eddy (Read and Pollard 2001).

^dCalculated from seven research cruises.

value should be compared with those given in Table 2. Using data from the Coastal Zone Color Scanner (CZCS) for the years 1979–1985 Brown and Yoder (1994a, p. 7479) calculated a mean annual areal extent of coccolithophore blooms in subpolar and polar water of $1.0 \times 10^6 \text{ km}^2$ (0.28% of the global ocean area). If we assume that this bloom area would last one month on average (Brown and Yoder 1994b) and constantly emit DMS at the high rate of $20 \mu\text{mol m}^{-2} \text{ d}^{-1}$, this would input $6 \times 10^{-4} \text{ Tmol}$ of sulfur to the air. This is equivalent to 0.12% of the annual biogenic sulfur input value of 0.5 Tmol a^{-1} estimated by Bates et al. (1992). The quantity of sulfur emitted is further reduced if we take the areal value for white

water as $4.42 \times 10^5 \text{ km}^2$, (range 3.5 to $5.81 \times 10^5 \text{ km}^2$, 44% of the CZCS value) calculated from more recent Sea-Viewing Wide Field-of-View Sensor (SeaWiFS) satellite data for 1997–2002 (E. Buitenhuis and C. Brown, pers. com. 2002). The cause of this reduction in apparent areal extent is currently under investigation.

Satellite based studies to assess whether DMS-producing blooms of *E. huxleyi* have any climatic effect have given mixed results. Falkowski et al. (1992) found that variability in low level cloud albedo for the central North Atlantic south of Iceland correlated with chlorophyll concentration and temperature, especially for the June–July period when *E. huxleyi* blooms occur in this area. In a more recent study Gondwe et al. (2001) used remotely sensed water-leaving radiances for the North Atlantic for 1998–1999 period and concluded that the coccolithophore blooms in this area had a negligible effect on direct radiative forcing of basin-scale climate.

To leave the discussion here would give a false impression because, as mentioned previously, the global distribution of *E. huxleyi* far exceeds the areal extent of blooms visible via satellite. A compilation of DMS flux data (Table 2) suggests that higher range emission rates can also be found in non-bloom areas. Coccolithophores are recognized as an important component of the tropical phytoplankton community (Haidar and Thierstein 2001). They can alter surface alkalinity (Bates et al. 1996) and pCO_2 in the Sargasso Sea, and add to the ‘ballast’ effect whereby their particulate organic material sinks and leads to carbon mineralization deep in the water column (the so-called CO_2 draw down effect). These observations emphasize the significance of coccolithophores in the carbon cycle and by extension it seems likely that they are also important for the sulfur cycle. However, more studies are needed to look at the microbial ecology of DMSP and DMS production in the open ocean to determine whether this differs from what is known for *E. huxleyi* blooms in the northern hemisphere.

DMS production in the past

It is instructive to examine the records of phytoplankton speciation and the trace components of the atmosphere in the geological past, because such data might aid us in predicting what might be seen with future changes in climate. The coccolith-bearing haptophytes have one of the best fossil records of any marine phytoplankton group. From the major floristic changes and variation in coccolithophore abundance seen in marine sediment cores the primary radiation of the group is thought to have occurred 230 million years ago in the Early Jurassic (Young et al. 1994). The dominant impetus for geological studies of fossil coccoliths has been to produce stratigraphic data (e.g. taxonomy and space and time distribution) to determine sediment age for the oil industry. Whilst such work obviously continues, increasing recognition of the importance of the coccolithophores for global biogeochemistry has led to studies which aim to relate detailed stratigraphic data with the paleoenvironment (Young et al. 1994). It is possible that coccolithophore speciation changes could have influenced DMS emissions to the early atmosphere

and may have contributed to paleotemperature fluctuations, but rather few studies have examined the coccolith sedimentary record as a potential proxy for DMS production. A notable exception is the study of Henriksson et al. (2000) which quantified coccoliths, alkenones and dinosterol in an equatorial Atlantic core and indicated that DMS production increased during glaciations. However, it should again be noted that because DMS is a product of the microbial ecology of the euphotic zone, the extrapolation between potential DMSP-containing phytoplankton and atmospheric DMS is not necessarily straightforward. Nevertheless, we suggest that as more information becomes available for DMS/P production by modern day coccolithophores, we will be in a much better position to exploit the full potential of this approach.

Arctic and Antarctic ice sheets contain a wealth of information on trace components of the atmosphere in past ages and the analysis of ice cores has greatly advanced knowledge of past climate change. The atmospheric oxidation products of DMS, sulfate and MSA, are readily removed from the air in wet and dry deposition via rain, snow and particles. The analysis of non-sea-salt sulfate and MSA in polar ice-cores gives a window on how atmospheric DMS levels, and by extrapolation water phase production of DMS, have fluctuated with time. MSA is particularly useful in this respect since it is considered to have no other significant air-phase sources. However, it must be emphasized that this is not a trivial task given the complex atmospheric chemistry of DMS (Ravishankara et al. 1997; Yin et al. 1990) and the considerable difficulties associated with the interpretation of ice-core data (Delmas 1995; Pasteur and Mulvaney 2000).

Recent Antarctic ice-core data covering four glacial cycles and a 420,000-year time span, suggests that DMS concentrations were about 5-fold higher during glacial periods (E. Saltzman, pers. com, 2000). Results for a Greenland ice core were not entirely in agreement with this since they gave the opposite MSA deposition pattern, but that for non-seasalt sulfate was similar. Higher ratios of MSA to non-seasalt sulfate were seen during warmer climate stages and the overall relationship with temperature was linear (Hansson and Saltzman 1993). These data may reflect how temperature influences the branching pathway that leads to MSA production during atmospheric oxidation of DMS, and they underline the complexity of using MSA as a quantitative proxy for DMS in ice cores.

The Antarctic ice-core record is in accord with higher marine primary productivity during glaciations, when stronger atmospheric circulation and concomitant changes in ocean circulation, higher nutrient inputs from deeper mixing and increased deposition of iron-rich dust to the oceans occurred. It also agrees with the Atlantic sediment core coccolith data mentioned previously (Henriksson et al. 2000). However, as discussed by Watson and Liss (1998) the resulting decrease in atmospheric CO₂ and increase in DMS would have led to further cooling and so a positive feedback on the climate system, not the negative feedback envisaged in the CLAW hypothesis (Charlson et al. 1987). From the phytoplankton perspective *E. huxleyi* is a 'young' species in geological terms – it first appeared in the fossil record for tropical waters about 270,000 years ago and became abundant ~80,000 years before present. It appeared to replace *Gephyrocapsa caribbeanica* (Thierstein et al. 1977). This is interesting given that the ice core data suggest significant

DMS production prior to 100,000 years ago and similar inferred DMS levels over the last four glacial cycles. While it is tempting to speculate that this suggests that *G. caribbeanica* has similar DMS production characteristics, we should quickly note that there is no evidence for this in the DMS literature. It is also important to acknowledge that ice cores do not necessarily give a snapshot of the past global atmosphere, rather Antarctic cores probably reflect processes going on within the area bounded by the Antarctic Circumpolar Current and its overlying atmosphere. Diatoms are usually considered the dominant phytoplankton group in the Southern Ocean, but a recent study shows that coccolithophores are numerically the major component of the flora in waters between 34° and 57° south, and *Emiliania huxleyi* was the dominant coccolithophore south of the Antarctic Polar Front (Eynaud et al. 1999). Winter et al. (1999) found living coccospheres of species that are considered to have a subtropical distribution in the Weddell Sea and suggested that the temperature tolerance of coccolithophores should be revisited. Studies of phytoplankton communities which combined CHEMTAX analysis of HPLC pigments and microscopy, show that haptophytes, including coccolithophores and *Phaeocystis antarctica*, are common in the Antarctic region (Wright and Van den Enden 2001).

DMS production in the future

Without the aid of a crystal ball it is hard to predict whether DMS emissions will alter significantly in the future! Nevertheless, useful insight can be gained from studies which consider past (see above), recent and projected changes in phytoplankton abundance, distribution and speciation, as well as studies addressing future DMS production more specifically. Modeling approaches for estimating possible DMS production under future climate scenarios were considered earlier in the chapter.

The availability of data from satellite sensors such as CZCS (October 1978 to June 1986) and SeaWiFS (since November 2001) has transformed our ability to observe and quantify ocean color, and revolutionized understanding of phytoplankton populations in the sea. In a recent paper Gregg and Conkright (2002) revised CZCS data to make it compatible with the SeaWiFS record and merged both >200 m water depth data sets to give a record spanning 1979 to 2000. Areas sparsely sampled by CZCS were omitted. They found similar global spatial chlorophyll distributions and seasonal variability for the whole period, but the record indicated an overall chlorophyll decrease of about 6%. Much of this reduction was related to reduced phytoplankton growth at high latitudes, whereas chlorophyll increased in the low latitude basins and little change was observed in the mid-ocean gyres. In more recent years warmer sea surface temperatures and reduced wind stress have resulted in shallower mixed layers leading to the reduced chlorophyll levels observed in high latitudes. The authors concluded that some of the decadal changes observed reflected the biota responding to changes in climate.

In the early 1930's before the advent of ocean-viewing satellites, regular monitoring of phytoplankton speciation and abundance at the ocean basin scale was initiated by deploying the Continuous Plankton Recorder (CPR) from merchant ships over extended transects through the North Atlantic and the North Sea. The survey still operates today and it maintains true continuity of sampling by using the same basic CPR design and analysis techniques. The samples which represent 3 m³ of seawater are analyzed for color index (a measure of phytoplankton biomass) and over 400 phytoplankton and zooplankton taxa are identified and counted (John et al. 2002). During the last decade most of the CPR regions, including the more southerly areas of the Atlantic and the North Sea, have shown an increase in phytoplankton abundance, but a decrease has been observed in the northern oceanic region of the Atlantic (Reid et al. 1998; Edwards et al. 2001). These decreases are thought to be due to changes in the North Atlantic Oscillation (NAO) which have shifted the center of deep-water convection from the Greenland Sea to the Labrador Sea from 1988 onwards.

In a recent study Iglesias-Rodríguez et al. (2002) used coccolithophore bloom satellite data and climatological maps for physical and chemical variables, to develop a method whereby the spatial and temporal distribution of such blooms could be predicted. They conclude that within this century the areal coverage of coccolithophore populations in the Northern Hemisphere will decrease by as much as 50%, whilst a 5% decrease is likely in the Southern Hemisphere. A laboratory study has shown that calcification would be reduced in *E. huxleyi* and *Gephyrocapsa oceanica* in response to the 750 p.p.m.v atmospheric CO₂ level predicted for future (Riebesell et al. 2000). Under these conditions the cultures produced a substantial proportion of malformed coccoliths, but photosynthetic carbon fixation was enhanced. It is not so unusual to find coccolithophores with some malformed coccoliths in seawater samples today, but it is not known whether an increased percentage of such coccoliths would reduce the niche for this phytoplankton group. We are not aware of any experiments that have considered DMSP and DMS production under the doubled CO₂ scenario. In the natural environment increased seawater pH alters the carbonate system and doubled CO₂ would seriously perturb the carbon cycle. This could reduce any competitive advantage that coccolithophore CO₂-concentrating mechanisms confer in the contemporary ocean, and might lead to changes in the composition of phytoplankton assemblages.

In addition to ongoing CO₂ and pH changes, alterations in the quantity of UV radiation, temperature changes, alteration to wind field patterns and concomitant changes in nutrient supply from upwelling zones and the atmosphere (e.g. iron deposited to the sea via dust), could all potentially alter phytoplankton biomass and speciation. As discussed previously, DMSP-containing phytoplankton are a necessary prerequisite for DMS production so any shift in phytoplankton assemblages towards higher or lower level DMSP species could be significant. For instance sea surface warming would decrease turbulence and tend to reduce the niche for diatoms and select for coccolithophores, but the decreased turbulence plus acidification could increase the niche for dinoflagellates. However, because DMS is a product of the interactions of the microbial food web it is hard to predict potential DMS production with knowledge of phytoplankton biomass and speciation alone.

As mentioned earlier the balance between bacterial sulfur demand relative to DMSP availability seems critical (Kiene et al. 1999), and UV radiation can reduce bacterial activity especially when the mixed layer depth is shallow (Simó and Pedrós-Alió 1999). Shenoy et al. (2002) found that MLD was a major control on water column DMS concentration in the Indian Ocean, but substantial interannual variability in DMS flux was seen which they suggested could be due to differences in windspeed and/or the composition of the phytoplankton assemblage. Marine phytoplankton generally have a better level of protection against UV than bacteria, so enhanced UV might favor DMS production via algal DLA over bacterial demethylation. Increased UV would also increase photooxidation of DMS to DMSO, and according to the antioxidant hypothesis of Sunda et al. (2002) cellular levels of DMSP and DLA might also increase.

Outlook

We must emphasize that the quantity of DMS that is emitted to the atmosphere is a very small fraction of the marine DMSP plus DMS pool. Essentially the flux is a small 'leak' from the DMS biogeochemical cycle (see Fig. 2). However, it is not hard to imagine scenarios where small changes in phytoplankton biomass, phytoplankton speciation, geographic distribution, grazing, bacterial activity, viral lysis etc could make a significant impact on DMS emissions with knock-on effects on climate.

More than eight years have passed since one of us wrote a chapter on DMS production for Green and Leadbeater's book on 'The Haptophyte Algae' (Malin et al. 1994). In the intervening years, research on all aspects of DMS has increased in pace, but it seems that many challenges still remain. A large proportion of the current understanding of marine DMS and DMSP production is still only derived from studies on cultures and natural populations containing *E. huxleyi* and *Phaeocystis*. Research on these key species should continue, but we assert that there is a clear case for additional projects focusing on other coccolithophores and non-calcified haptophytes, and process studies in open ocean situations where phytoplankton biomass is relatively constant – the 'non-bloom' situation. The common conjecture is that all haptophytes produce significant quantities of DMS, but as discussed here this assumption is based on a rather scant data set. Returning to the title of this chapter we feel that the evidence collated here suggests that the contribution that coccolithophores (calcified and non-calcified cells) make to total production of DMS in marine waters could be a substantial proportion of the whole, though more in-depth studies are needed to confirm this. Some current modeling initiatives are based on separating marine phytoplankton into functional groups or biogeochemical guilds i.e. organisms that are related through common biogeochemical processes rather than being genetically related. Given current knowledge, when predicting global and regional DMS production or carbon cycling there is a tendency for *E. huxleyi* to be considered the 'typical' DMS producer and/or typical coccolithophore. While this may prove to be the case, we assert that

this assumption needs to be tested experimentally in the laboratory and in the field. Additionally, it would also be worthwhile to pay greater attention to other phytoplankton groups such as the dinoflagellates and diatoms, which have received little research attention in the past. In this way a broader and clearer picture of the production of DMSP and DMS by marine phytoplankton should emerge.

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Re-evaluation of the physiological ecology of coccolithophores

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Summary

Recent data on coccolithophore abundance and calcification from the Atlantic, Pacific and Indian Oceans is interpreted within the traditional physiological ecology paradigm known as the “Margalef Mandala” (Margalef 1978). As predicted by Margalef, coccolithophores should be most abundant in conditions of moderate turbulence and nutrient concentrations. However, blooms of coccolithophores (and other algal groups, too) show day length preferences, suggesting that the Margalef Mandala might be expanded to include day length as a third dimension. Variability in calcification per cell makes it difficult to extrapolate coccolithophore abundance (in the Margalef Mandala) to carbon fixation and vertical carbon fluxes. Because of this, there is a discrepancy between biologists and geologists concerning when coccolithophores should be most abundant. Biologists contend that coccolithophores are found in stratified, mesotrophic, environments while geologists contend that they are most abundant in highly productive environments. A conceptual model is presented which attempts to bridge the two schools based on a) variable calcification per cell, and b) grazing differences between mesotrophic and eutrophic environments that will alter the vertical flux of the coccolithophores to the sediments.

Introduction

Traditional views of coccolithophore ecology have been firmly rooted in the seminal work of Margalef (1978). He suggested that, within the two dimensional phytoplankton niche space defined by turbulence and nutrients (“Margalef’s Mandala”), coccolithophores fell between the diatoms (which exploit well-mixed, high nutrient regimes) and the dinoflagellates (which exploit stratified, low-nutrient regimes). Margalef’s paradigm extended to growth strategies, too, based on nutrient

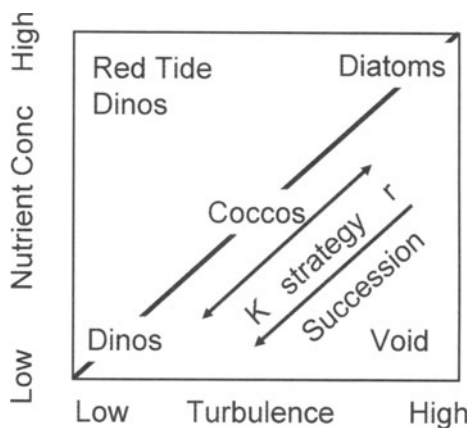


Fig. 1. Re-drawn version of Margalef's mandala (Margalef 1978) (his Fig. 2), illustrating the two-dimensional niche space model of diatoms, dinoflagellates and coccolithophorids relative to turbulence and nutrients.

uptake kinetics. He suggested diatoms to be fast-growing “r” strategists, dinoflagellates to be slower-growing “K” strategists, and coccolithophores to be somewhere between “r” and “K”. Margalef suggested that these functional groups appear following a mixing event, typically showing succession from the diatom-dominated communities, to coccolithophores, ultimately to dinoflagellates (Fig.1). It is important to note that other algal groups, such as cyanobacteria, picoeukaryotes, blue-green algae, and prochlorophytes, were not included in the mandala (some of these algal groups were not even known at the time of Margalef's work). Nonetheless, based on the algal classes that he described, we would expect highest coccolithophore abundance, suspended calcite concentration, and calcification in environments of moderate turbulence and nutrients. This paper, although not meant to be an exhaustive review of all coccolithophore ecology, will address the applicability of Margalef's paradigm to some selected recent coccolithophorid observations.

In discussing the niche of coccolithophores relative to diatoms and dinoflagellates, it is important not to overlook some of the details of species distribution described by others. Guillard and Kilham (1977) commented on the importance of cell size in its relation to surface-to-volume ratios and growth rate. They specifically focused on diatoms but their discussion is equally applicable to other algal classes. Moreover, their analysis of the different types of diatoms is somewhat at odds with the Margalef (1978) paradigm since they described specific large diatom species that are more characteristic of oligotrophic, low turbulence environments, as opposed to high turbulence environments. They also highlighted the fact

that algal succession can be strongly modulated by grazing pressure. The importance of grazing was also discussed by Margalef (1978).

Similar to the Guillard and Kilham analysis of diatom ecology, Young (1994) defined three ecological communities of coccolithophores, associated with three distinct environments: placolith-bearing cells such as *Emiliania huxleyi*, found in coastal or mid-ocean upwelling regions and umbelliform cells such as *Umbellosphaera tenuis*, found in more oligotrophic, nutrient-depleted waters. The last group, floriform cells (such as *Florisphaera profunda*), were associated with deep photic-zone assemblages in low to mid-latitudes. Moreover, Young discussed the appearance of motile cells within the placolith-bearing group; flagellae could substantially enhance the ability of coccolithophores to exploit stratified, nutrient-poor environments. Similar to Margalef's predictions of coccolithophore growth strategies, it is likely that the three groups of coccolithophores defined by Young, show differences in their growth strategies which ultimately would relate to their natural abundance and production of calcium carbonate (otherwise known as particulate inorganic carbon, PIC). Interestingly, it still is not known whether conceptual models such as Young's and Margalef's predict not only coccolithophore abundance in nature, but also their rates of carbon fixation via photosynthesis or calcification. Satellite observations of mesoscale *E. huxleyi* blooms (starting with Holligan et al. (1983)) illustrated what marine geologists had long-since known, that coccolithophore calcite was an important component of the marine carbon cycle. These satellite observations gave rise to new research on coccolithophore blooms and calcification rates, under the auspices of the International JGOFS (Joint Global Ocean Flux Study). There has been a profound need to synthesize field observations of coccolithophores in order to link traditional taxonomic enumeration studies to the more process-oriented C studies.

Synthesis of field observations from three oceans

Pacific Ocean

Coccolithophore observations in the Pacific have consisted mostly of microscope enumeration of seawater samples and sediment trap samples (Okada and Honjo 1973, 1975; Okada and McIntyre 1977, 1979; Reid 1980) with considerably less attention devoted to coccolithophore growth and carbon fixation. One study which addressed coccolithophore abundance and their carbon fixation was conducted in the Equatorial Pacific during the summer of 1992, in which stations were run along 140°W, from 1°N to 12°S (Balch and Kilpatrick 1996). This study focused on both plated coccolithophore cells (coccospheres) and their detached coccoliths. This discrimination is made due to the profound effect each can have on optical scattering of the water column (Balch et al. 1991; Voss et al. 1998); calcite-specific light scattering is much higher for the detached coccoliths than intact coccospheres. A striking observation from this survey was the confirmation of a dramatic decrease in birefringent detached coccoliths below 60 m depth. Okada

and Honjo (1973) also observed decreases in coccolithophore cell abundance by as much as 30–40 x over the top 200 m in the Equatorial region. Reid (1980) cited significant decreases in the numbers of coccolithophore cells with depth in the North Pacific Gyre, as well. The 1992 study demonstrated a strong association of coccoliths with the Equatorial Current, with lower, but still significant, coccolith concentrations in the South Equatorial Counter Current (SECC) and North Equatorial Counter Current (NECC) (Fig. 2). Okada and Honjo (1973) observed a similar enhancement of coccolithophores in the equatorial region between 12°S and 5°N, but their contouring intervals did not allow demarcation of the specific current regimes of the Equatorial Pacific.

Equatorial Pacific patterns of primary production, calcification and calcite standing stock did *not* match the above patterns of coccolithophore abundance (Balch and Kilpatrick 1996) (Fig. 3). For example, primary production (performed by coccolithophores and non-coccolithophore algae alike) not surprisingly showed a much broader mesoscale pattern with a maximum near the equator and gradual decreases with increasing latitude. However, calcification rates were more patchy, with a maximum just to the south of, but directly adjoining, the diatom-dominated productivity maximum between the equator and 2°N. This diatom community “outcropped” at the surface at the 2°N convergence front, producing the “Line in the Sea” feature (Archer et al. 1997; Yoder et al. 1994). Adjacent coccolithophore and diatom communities have been observed in other settings and will be discussed more later. The concentration of PIC was highest, directly under regions of highest calcification (Fig. 3). (Note, the PIC in this example likely was comprised mostly of coccoliths, not foraminifera and pteropod tests, purely due to the statistics of the sampling. This was due to the small sample volumes used in the PIC analyses. That is, the probability of capturing a foraminifera or pteropod – typically found at concentrations of a few individuals m⁻³ – in the one-liter PIC samples, was negligible.) High PIC directly under regions of high calcification is suggestive of grazing and repackaging of the coccoliths into larger, faster-sinking particles. But the fact that highest PIC concentrations generally did not extend below 100 m suggests that some other processes were involved in the removal of the PIC. Given the Equatorial Pacific patterns of calcification, a comparison with total carbon fixation showed that, on an integral basis, coccolithophore calcification represented ~3–12% of the total carbon fixation, with peaks at the equator and in more oligotrophic waters found at latitudes exceeding 7°N or S (see Fig. 7 of Balch and Kilpatrick (1996)). Estimates of PIC turnover based on its standing stock divided by its production rate were typically 2–10 d. The fact that coccolithophore calcification was highest in Equatorial waters adjoining diatom-dominated communities, certainly is consistent with the Margalef Mandala paradigm, although association of coccolithophores with a region of strong upwelling is not predicted *a priori*. It is important to caution, however, the fact that these Equatorial Pacific calcification data are limited to one season and thus do not allow insight into temporal evolution of the communities.

Another Pacific observation was consistent with predictions based on Margalef’s mandala (Margalef 1978). During the 1990’s, spectacular coccolithophore

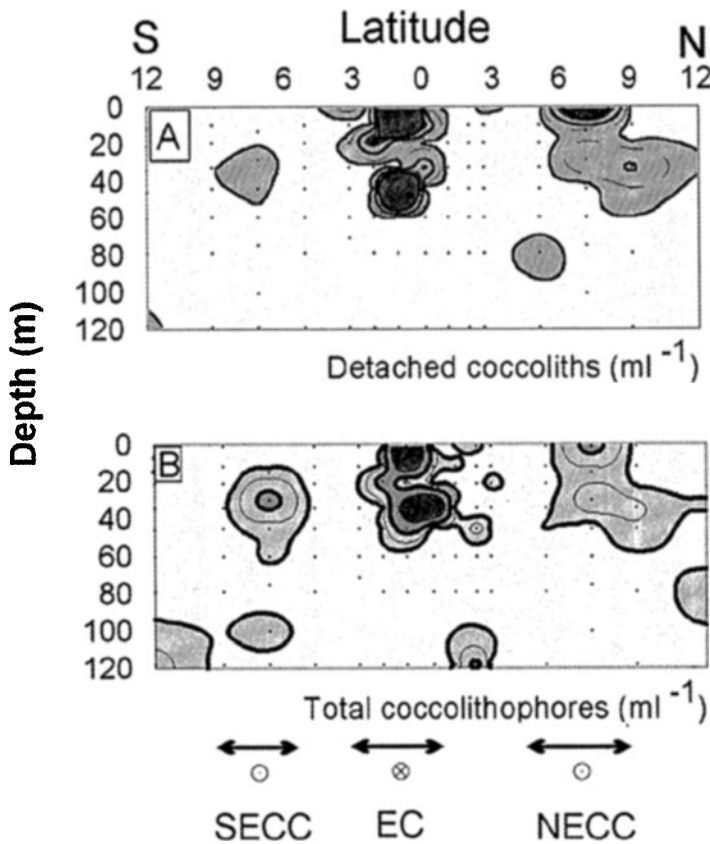


Fig. 2. A. Cross section of coccolith concentration (showing coccoliths detached from coccospheres) across equatorial Pacific Ocean at 140°W from 12°N to 12°S during August and September 1992 (re-contoured from data in Fig. 1 of Balch and Kilpatrick (1996)). Coccolith concentration is designated with darkening shades of gray with the following values, respectively: <50 ml⁻¹ (lightest), 50–200 ml⁻¹, 200–400 ml⁻¹, >400 ml⁻¹ (darkest). B. Section showing concentration of coccospheres along same section in panel A. Coccolithophore concentration is designated with darkening shades of gray with the following respective values: <5 ml⁻¹ (lightest), 5–9.99 ml⁻¹, 10–40 ml⁻¹, and >40 ml⁻¹ (darkest). Re-contoured from the data in Fig. 2 of Balch and Kilpatrick (1996)). SECC designates the South Equatorial Counter Current (flowing eastward, here shown as a circle with dot in the middle), EC designates the Equatorial Current (flowing westward, shown as a circle with “X” inside), and NECC designates the North Equatorial Counter Current (flowing eastward, again shown as a circle with dot in the middle), with vertical dotted lines indicating their latitudinal extent.

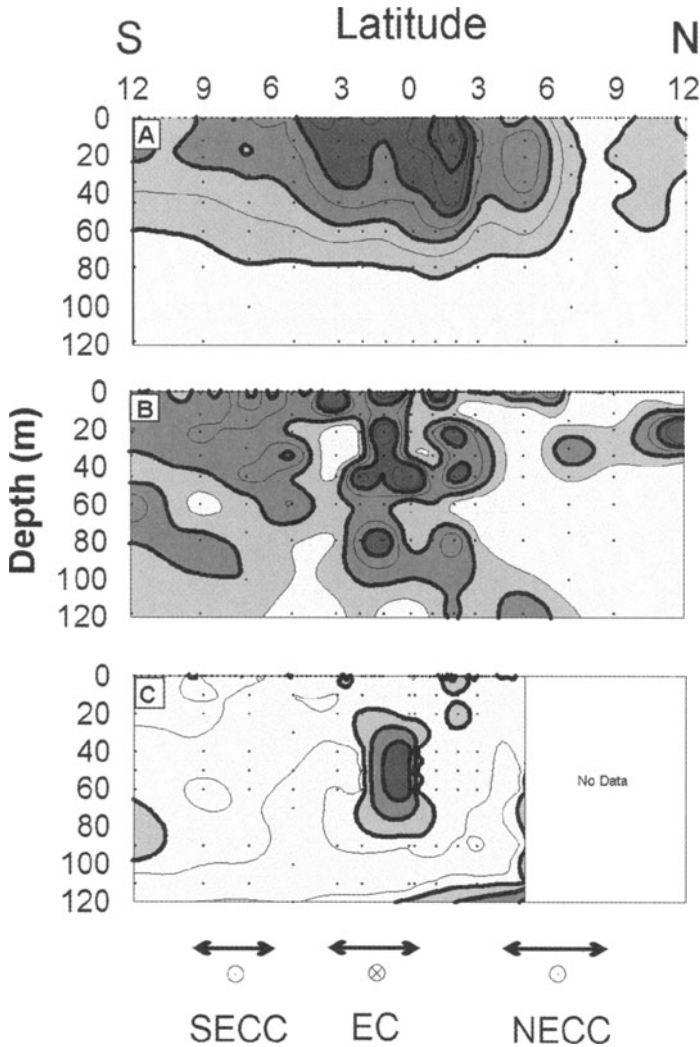


Fig. 3. Vertical sections along 140° W for photosynthesis, calcification and PIC, recontoured from data in Fig. 4 of Balch and Kilpatrick (1996). Other details of sections are as described in Fig. 2. **A.** Photosynthesis-contour levels are 2, 5, 8, 11, 14, 17, 20, 23, and 26 mg organic C m⁻³ d⁻¹. Contour shading corresponds to courser gradations: <2 (lightest), 2–8, 8–14, and 14–20 mg organic C m⁻³ d⁻¹ (darkest). **B.** Calcification-contour levels are 0.2, 0.4, 0.6, 0.8, and 1 mg calcite C m⁻³ d⁻¹. Contour shading corresponds to courser gradations: <0.2 (lightest), 0.2–0.4, and 0.4–0.8, >0.8 mg calcite C m⁻³ d⁻¹ (darkest). **C.** Suspended PIC-contour levels are 0, 2, 4, 8, and 16 mg PIC m⁻³. Contour shading corresponds to courser gradations: <4 (lightest), 4–8, 8–16, >16 mg PIC m⁻³ (darkest). Currents and their directions designated as in Figure 2.

blooms were observed in the Bering Sea, previously not seen before in remote sensing images. The blooms generally appeared brightest in late summer, although turbid water features have been noted in SeaWiFS images as early as February. It now appears that the February events were merely sediment resuspension events over the shallow Continental Shelf region (Broerse et al. 2003). The other mesoscale features in the Bering Sea were indeed coccolithophore blooms, and appeared to be associated with anomalously warm North Pacific conditions and stratification of surface waters (Napp and Hunt 2001).

Indian Ocean (Arabian Sea)

The only comparable measurements of Indian Ocean coccolithophore abundance and calcification are the JGOFS measurements of Balch et al. (2000). The important temporal variable in the northern Indian Ocean is the intense wind forcing associated with the SW Monsoon during the summer and NE Monsoon during the winter (with intervening intermonsoon periods). Abundance of coccolithophores and detached coccoliths (integrated to the base of the euphotic zone) showed 20% higher abundance during the more stratified intermonsoon, but the difference was not significant. Spatially, this translated to highest abundance offshore, with some moderate increases in areas affected by coastal upwelling. In terms of PIC concentrations, there was ~10% more PIC overall during the SW Monsoon, but again, such differences were insignificant. Turnover times of integrated PIC were between 8–19 days and were not very different from the particulate organic carbon (POC) turnover times; this was not expected given the lack of nutritive value in calcite. It suggests that PIC is not discriminated from POC during ingestion and grazing. Unlike cell abundance and PIC, calcification showed a highly significant doubling during the SW monsoon. This raises the interesting possibility that, during the SW monsoon, there is increased calcification in the coastal zone, performed by fewer coccolithophore cells. Indeed, calcification normalized to the concentration of plated coccolithophore cells (cocospheres) was 7 x greater during the SW Monsoon, the period of *increased* mixing (Fig. 4). While this analysis does not take into account any differences in coccolithophore species composition between the two sampling periods, the observations clearly were not predicted from the Margalef Mandala, *a priori*.

Atlantic Ocean

The Atlantic Ocean (including its northern marginal seas) has had some of the most spectacular coccolithophore blooms ever observed, and by far the most measurements of calcification and coccolithophore abundance. The regions of the Atlantic most cited for blooms are the region south of Iceland (Holligan et al. 1993; Robertson et al. 1994; Balch et al. 1996a; Balch et al. 1996b; Tyrell and Taylor 1996), the North Sea shelf and shelf-break waters (Birkenes and Braarud

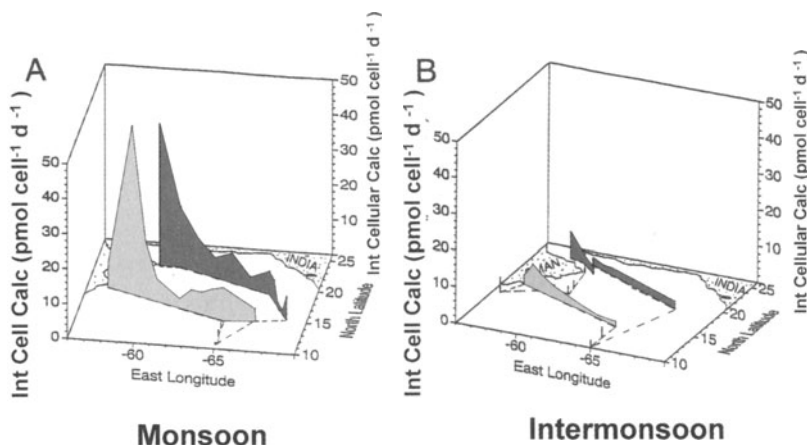


Fig. 4. Integrated calcification rate normalized to the integrated concentration of plated coccolithophore cells (coccospheres) in the Arabian Sea during **A.** SW Monsoon and **B.** Intermonsoon periods (redrawn from Balch (2000)). Northern and southern survey transects are shown in dark gray and light gray, respectively. Cruise mean cellular calcification rate was $17.4 \text{ pmol cell}^{-1} \text{ d}^{-1}$ for the monsoon and $2.5 \text{ pmol cell}^{-1} \text{ d}^{-1}$ for the intermonsoon. They were significantly different with a significance level of $P < 0.05$.

1952; Berge 1962; Holligan et al. 1983; Groom and Holligan 1987; Sournia et al. 1987; Van Bleijswijk et al. 1991; Garcia-Soto et al. 1995; Van der Wal et al. 1995; Brussaard et al. 1996; Buitenhuis et al. 1996; Marañón and González 1997; Wilson et al. 2002), and coastal shelf waters of the NW Atlantic (Balch et al. 1991; Balch et al. 1992; Brown and Yoder 1993; Matrai and Keller 1993; Brown and Yoder 1994b; Graziano et al. 2000;). Blooms have also been observed off the Argentine continental shelf in the South Atlantic (Brown and Yoder 1994a) and off of South Africa (Mitchell-Innes and Winter 1987). Detailed descriptions of north Atlantic coccolithophore assemblages have also been done (McIntyre and Bé 1967; Okada and McIntyre 1977, 1979; Kleijne 1991; Haidar and Thierstein 2001). Observations of coccolithophore calcification in non-bloom situations, such as the Gulf of Maine observations of Graziano et al. (2000), are not as common.

It is important to point out some potential biases in coccolithophore observations, particularly in the Atlantic given the frequency of turbid blooms. Optical observations of coccolithophores are biased to visible blooms, and the species most responsible for the turbid blooms is *Emiliania huxleyi*. These blooms are fairly common in the north Atlantic during summer months. This bias is because the coccoliths of this species are quite small, and the amount of scattered light per gram of calcite is high (i.e. the coccoliths have a large scattering cross-section (Balch et al. 1996a)). Thus, other coccolithophore species are not as likely to form

visible turbid blooms. This can cause an apparent discrepancy between the coccolithophores that we “see” in the water column, and those that geologists find in the sediments (see later discussion on this disparity). Nonetheless, field observations of coccolithophore calcification are not subject to this bias, since those measurements are based on bulk incubations which represent the calcification of all calcifying species, not just *E. huxleyi*.

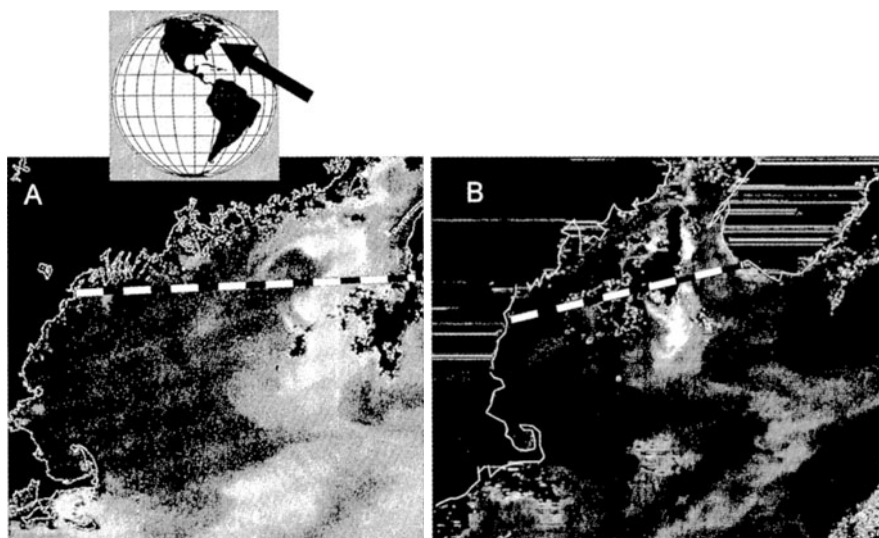


Fig. 5. A. SeaWiFS image of relative CaCO_3 concentration in Gulf of Maine (20 June, 2000) during a small *E. huxleyi* bloom. Data processed using Gordon et al. (2001) three-band algorithm. The lighter shades of gray represent areas of higher PIC concentration. Inset globe shows the location of the Gulf of Maine. **B.** MODIS image of absolute PIC concentration in Gulf of Maine from June 20, 2000, using the two-band PIC algorithm. The basis of this algorithm is described in Gordon et al. (1988) and required calcite-specific backscattering coefficients for *E. huxleyi* can be found elsewhere (Balch et al. 1996a, 1996b, 1999). Note, the MODIS image was taken earlier than the SeaWiFS image on the same day. The patch of clouds that obscured the center of the bloom in the MODIS image did not obscure the patch center in the SeaWiFS image, allowing the observation of its horseshoe shape. The advection of coccoliths around northern flank of Georges Bank is also apparent in the MODIS image, with fine-scale eddy structure along the frontal boundary. Scale-White = 3×10^{-3} moles PIC m^{-3} ; light gray = 2×10^{-3} moles PIC m^{-3} ; dark gray = 0.75×10^{-3} moles PIC m^{-3} ; black = $0-0.1 \times 10^{-3}$ moles PIC m^{-3} . Ferry cruise track used to collect the data of Fig. 6 is shown with dashed black and white line in both panels.

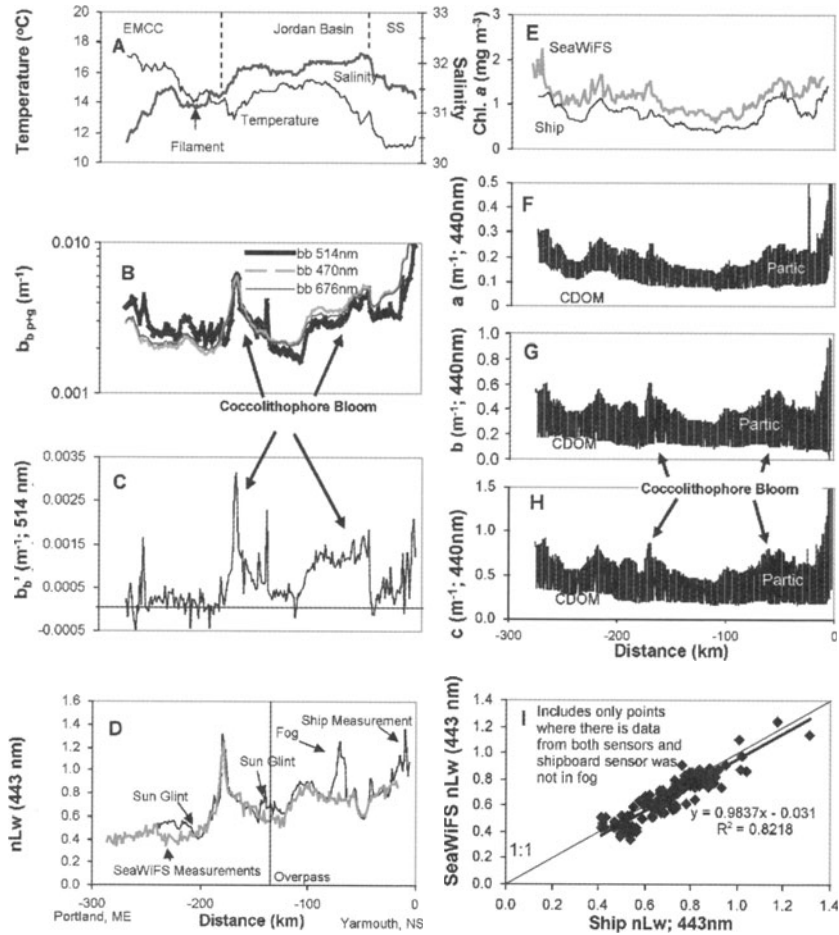


Fig. 6. Semi-continuous data taken from July 2, 2000 transect through Gulf of Maine *E. huxleyi* bloom during its peak. Distance from Yarmouth, NS is on the X axis for panels A–H. **A.** Surface temperature and salinity. The eastern Maine coastal current (EMCC), Jordan Basin, and Scotian Shelf (SS) water are indicated. **B.** Backscattering measured by the Wyatt light scattering photometer (514 nm; based on 15 point volume scattering functions) and Hydrosat II (470 and 676 nm; based on volume scattering at one angle). Water values have been subtracted, thus b_b values are from particulate and dissolved material (Balch et al. 1999). It can be seen that the Wyatt data show higher frequency variability than the HSII, which is due to the smaller viewed volume (9 ml vs. 20 l). Moreover, both instruments clearly show similar trends, but their relative values vary, possibly due to changing shape of the volume scattering function (which is not taken into consideration with the HSII, but is with the Wyatt). The coccolithophore bloom is plainly visible in the backscattering on ei-

Fig. 6. (cont.)

ther side of the stratified Jordan Basin (60 and 170 km). **C.** Acid-labile backscattering at 514 nm (b_b' ; measured with the Wyatt instrument), due to coccolith CaCO_3 . Values in coastal current are not significantly different from zero (FlowCAM showed the algal assemblage consisted of diatoms and dinoflagellates, with few *E. huxleyi*). The ship moves 1.2 km during each 4 minute acidification cycle. Occasional negative b_b' values can result if phytoplankton b_b values *increase* (due to horizontal variability) during an acidification cycle (whereas b_b values should *decrease* due to CaCO_3 dissolution). In the coccolithophore bloom, calcite b_b' represented ~50% of the total backscattering at 514 nm (compare with panel C). **D.** radiometer data for normalized water-leaving radiance at 443 nm (nLw; $\text{mW cm}^{-2} \mu\text{m}^{-1} \text{sr}^{-1}$) compared with SeaWiFS values ($\text{sr} = \text{steradian}$). At 60 km, the ship passed through a fog bank, causing higher nLw values. Significantly increased nLw in the coccolithophore bloom was evident. **E.** Fluorescence- and SeaWiFS-derived (OC-4 model; O'Reilly et al. 1998) chlorophyll concentrations. One can see that the OC-4 algorithm generally overestimates chlorophyll by $\sim 0.25 \mu\text{g l}^{-1}$ in the Gulf of Maine. **F., G., H.** Inherent optical property data on absorption (a), scattering (b), and attenuation (c) taken with an ac-9 absorption and attenuation meter. Data shown here have had pure water values subtracted. The black region represents particle IOPs (all particles $\geq 0.2 \mu\text{m}$ filtered), and the white region below represents the cDOM IOPs (water filtered through $0.2 \mu\text{m}$ poresize filters). One can see the increased cDOM absorption, scattering, and attenuation in the Eastern Maine coastal current. Also apparent in Jordan Basin is that 1/2 of the absorption, and 1/3 of the scattering is from cDOM. Scattering and absorption peaks were clearly evident in the *E. huxleyi* bloom. **I.** SeaWiFS vs. ship-measured nLw 510 nm ($\text{mW cm}^{-2} \mu\text{m}^{-1} \text{sr}^{-1}$) for data inside and outside coccolithophore bloom. Least-squares fit to the data has a slope that is not significantly different from 1.0.

There are a few unifying aspects of North Atlantic coccolithophore bloom observations (almost exclusively *E. huxleyi*). Turbid coccolithophore suspensions usually appear during May through July (within a month of the summer solstice, while surface waters are still heating and stratifying (Holligan et al. 1983; Keller et al. 1992; Townsend et al. 1994; Van der Wal et al. 1995)). Organic biomass associated with the blooms is usually low. This was especially well shown in the N. Atlantic *E. huxleyi* bloom of 1991 (Fernández et al. 1993; Holligan et al. 1993). Even in non-bloom situations, calcification: photosynthesis ratios peak at 15% at chlorophyll levels $< 0.5 \mu\text{g l}^{-1}$ (Graziano et al. 2000). Moreover, calcification associated with the ephemeral *E. huxleyi* blooms, as spectacular as they may be in satellite images, is of lesser significance globally due to their short 2–3 week duration (Balch et al. 1992; Graziano et al. 2000). Non-bloom calcification usually is between 1–3% of photosynthetic rates (Marañón and González 1997; Graziano et al. 2000), which, extended over time and space scales of the world ocean, likely accounts for more carbon fixation into CaCO_3 .

Anatomy of an *E. huxleyi* coccolithophore bloom

Many coccolithophore observations have been made in a Gulf of Maine phytoplankton sampling program, now in its 7th year, in which samples are taken from a ferry that travels between Yarmouth, Nova Scotia, Canada, and Portland, Maine, USA. A complete description is given in Balch (Balch 2001) and Balch et al. (submitted). While most *E. huxleyi* observations have been made during non-bloom situations, a small *E. huxleyi* bloom occurred in June 2000 that offered an ideal opportunity to sample and compare ship and satellite results (Fig. 5). The horse-shoe shaped bloom occurred at a frontal boundary (Fig. 6A), extending around the Jordan Basin within the Gulf of Maine, and the surface coccoliths were advected offshore, around the northern flank of Georges Bank. The feature

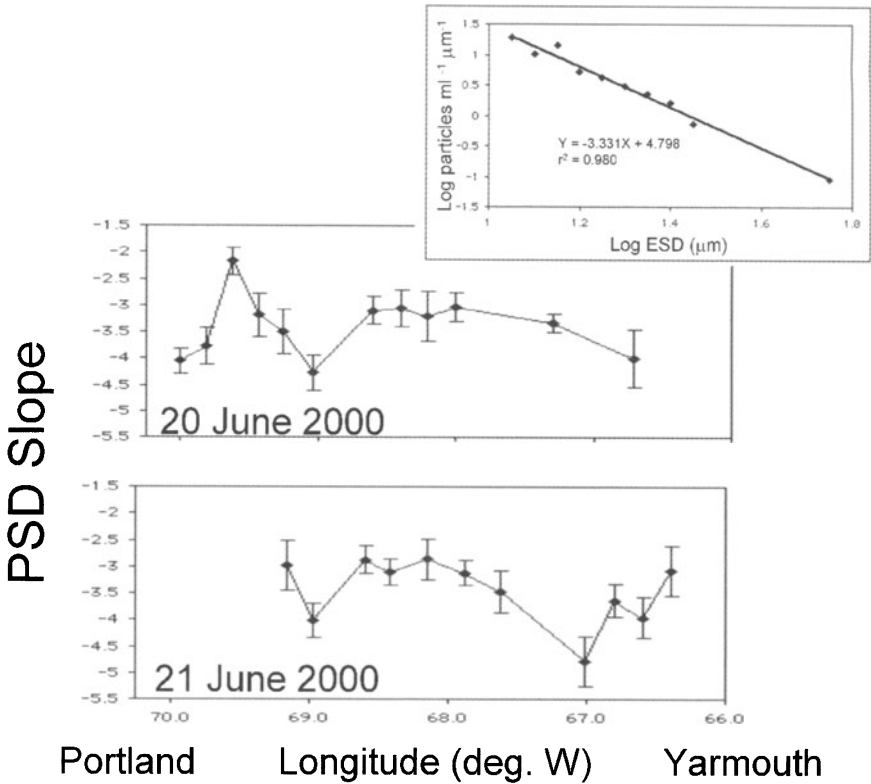


Fig. 7. Slopes of size distribution functions for each half-hour segment of Gulf of Maine ferry crossing as measured with Flow-Cam. Measurements were made on two consecutive days, 20 and 21 June 2000. Slopes were normalized to the bin size, according to Gin et al.(1999). Inset shows an example of a typical 1/2 hour Flow-Cam sample (sample from 6/20/00, 1146–1215 h EDT) from which the slope and error limits on the slope are calculated.

was identified by strong backscattering peaks (Fig. 6B and C), and was associated with high water-leaving radiance values (as measured either from the ship's bow or from SeaWiFS; Fig. 6D). Chlorophyll concentrations (estimated from ship and satellite) showed an offset of $0.25 \mu\text{g Chl a l}^{-1}$, but were well-correlated nonetheless (Fig. 6E and I). Inherent optical properties (IOPs) of total scattering, absorption and attenuation showed well-defined peaks associated with the bloom. The absorption, scattering and attenuation of the dissolved material of $<0.2 \mu\text{m}$ (colored dissolved organic matter; cDOM) showed the biggest increases in the Eastern Maine Coastal Current, on the western side of the transect near Portland (outside of the *E. huxleyi* bloom). Nonetheless, careful inspection of Fig. 6G shows subtle increases in cDOM scattering in the coccolithophore bloom. This has never been reported before. It is tempting to speculate that it resulted from release of cDOM during grazing on the algae (Eppeley et al. 1981) viral infection of the coccolithophores (Bratbak et al. 1993, 1995, 1996; Brussaard et al. 1996) or exudation of cDOM as cells within the bloom senesced. Such cDOM release has been observed from infected photosynthetic prokaryotes (Balch et al. 2002b)). The size distribution of the algal community demonstrated good reproducibility for consecutive days (Fig. 7); the most negative slopes of the particle size distribution were associated with small diatoms, just east of the *E. huxleyi* bloom, centered between $67.0\text{--}67.3^\circ\text{W}$. The region of diatoms within the eastern coccolithophorid patch was associated with a slight depression in the 550 nm normalized water-leaving radiance (Fig. 6D).

Assembling all of the Gulf of Maine ferry data from 2000, it is apparent that the *E. huxleyi* bloom was confined to the Jordan Basin Frontal boundary, and was most intense near the end of June and early July. A 3-axis plot of total *E. huxleyi* coccolith concentration versus daylength and water density (in sigma-theta units) illustrates exactly how narrow the space-time constraints were for this *E. huxleyi* bloom (Fig. 8). For example, peak *E. huxleyi* coccolith concentrations were associated with day lengths approaching 16 h (at the summer solstice), and moderate densities (between low density coastal waters and higher density Jordan Basin water).

It could be fortuitous that *E. huxleyi* blooms generally happen close to the summer solstice for their respective hemisphere (i.e. due to the correct physical conditions of temperature and stratification). This seems unlikely, however. Cultures of *E. huxleyi*, in which other physical conditions are held constant, are day length saturated at 12–16 h of light per 24 h day (Paasche 2002). Indeed, our own culture studies have used a 14:10 light : dark cycle due to better growth of the clones (Balch et al. 1993).

The above parameterization of the *E. huxleyi* bloom to density and day length is, however, consistent with Margalef's Mandala (Margalef 1978) and it provides the possibility of extending it to include seasonal phytoplankton growth preferences. Indeed, the striking association of the Gulf of Maine *E. huxleyi* bloom to moderate surface density water provides indirect confirmation of its stability preference. (Note, we show elsewhere (Balch et al. submitted) that the density of

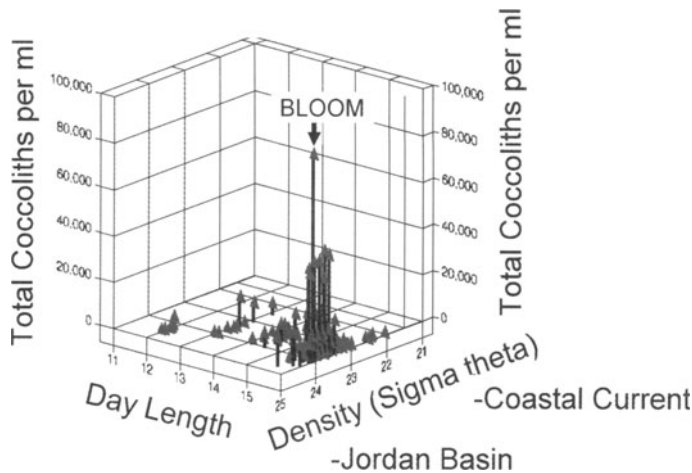


Fig. 8. Three-dimensional plot of total *E. huxleyi* coccolith concentration versus daylength (in hours) and seawater density. This Gulf of Maine coccolithophore bloom reached its maximum coccolith density on 21 June 2000, when daylength approached 16 h.

surface water in the Gulf of Maine is well correlated to the vertical temperature gradient in the top 50 m. Thus, the density axis in Fig. 8 is really indicative of vertical stratification). It could be argued that such *E. huxleyi* blooms occur in regions and times of moderate turbulence (and associated nutrient conditions). Moreover, it is well-known from culturing studies of phytoplankton (not just coccolithophores) that, all other things being equal, phytoplankton growth rates respond to day length, and that daylength is probably as important as light intensity (Brand and Guillard 1981). There are clear examples of species (e.g. *Ditylum brightwellii*) which, given sufficiently high light intensities, can grow close to their maximum growth rate with a 10 h day (Paasche 1968; Eppley 1977). Moreover, the period of the spring diatom bloom occurs during March and April in the North Atlantic, with day lengths of about 10–12 h. Laboratory and field results suggest that diatoms are, at least, capable of exploiting these relatively short day lengths with high growth rates. Observations on day length preferences of dinoflagellates are rare. Brand and Guillard (1981) demonstrated that certain oceanic dinoflagellate species either required, or were favored by, a dark period. For dinoflagellate culturing, Guillard and Keller (1984) commented that, “light-dark cycles of 16–8, 14–10 and 12–12 hr are commonly used”. Nonetheless, there are several observations of temperate dinoflagellate red-tides favoring longer day lengths (Yentsch et al. 1975; Nielsen 1992). For coccolithophores, experiments with *E. huxleyi* demonstrated that it had maximal growth rates at daylengths >16 h (Paasche 1967), consistent with Fig. 8. The response of *E. huxleyi* to day length may not be typical

of all coccolithophores, however. An oceanic coccolithophore species, *Gephyrocapsa oceanica* grew at a higher growth rate in a 14 h:10 h light: dark regime than in continuous light (Brand and Guillard 1981). Indeed, Brand and Guillard suggested that, of some 22 phytoplankton clones examined, most oceanic clones grew more slowly in continuous light than in a 14 h:10 h light: dark regime. Given these observations of daylength effects on phytoplankton growth, as a conceptual extension of Margalef's Mandala, it seems reasonable that the two-dimensional mandala could be given a third axis of daylength (Fig. 9), as a way to a) better include *E. huxleyi* blooms (Fig. 8) and b) provide differentiation for other species not yet included which likely will have their own day length preferences (e.g. cyanophytes, cryptophytes, prochlorophytes and the wealth of other picoplankton species). Day length could be considered one of several proximate (i.e., mechanistic) factors determining phytoplankton succession, *sensu* Guillard and Kilham (1977). Nonetheless, day length cues for growth would be subject to strong evolutionary selective pressures since changes in day length would help an organism unambiguously identify specific seasons (with associated physical and chemical conditions). In emphasizing the potential importance of day length on phytoplankton community succession, the reader should not overlook the potential importance of other factors such as grazing as a mechanistic control on succession and bloom formation.

Use of optics to assess the global distribution of suspended coccoliths

A major advantage for the detection of coccolithophores is the strong light scattering properties of their coccoliths. Calcium carbonate shows negligible absorption, but significant backscattering, of visible light (Balch et al. 1991). Thus, remote sensing can be used in ways not possible for other algal classes: knowing something about the physiological ecology of these organisms, plus their high visible-band reflectance, has allowed almost unambiguous identification of blooms from space. There are some important exceptions, however, that can cause ambiguities in the optical interpretation of coccolithophore features. First, the light scattering is mostly due to the calcite coccoliths, whether or not they are attached to a coccosphere. High blue-green reflectance does not necessarily indicate high coccolithophore organic biomass. In fact, the term "bloom" is a misnomer for coccolithophores given that organic biomass is usually lower than in blooms of other algal species. It also has been shown that the light scattering properties of a coccolithophore suspension will be different depending on the ratio of loose coccoliths and plated cells (Balch et al. 1991; Voss et al. 1998). Thus, while plated coccolithophore cells have strong unique optical signatures, "naked coccolithophores" can be found in the sea and cannot be distinguished from other algal functional groups by remote sensing.

Another important issue for the optical characterization of coccolithophores is that coccoliths from different coccolithophore species will not scatter light to the same

degree. It can be easily shown using calcite spheres, that the mass-specific scattering coefficient for calcite will be much lower for large calcite particles than small (Balch et al. 1996a). In nature, this means that foraminifera or pteropods will scatter light orders of magnitude less than the same mass of *E. huxleyi* coccoliths. Interestingly, based on Mie theory, the diameter of *E. huxleyi* coccoliths (~2 μm) optimizes their potential for scattering blue-green light (which also has fascinating evolutionary implications!). The remote sensing ramifications of this observation are that, on the one hand, it prevents mis-classification of foraminifera or pteropod suspensions as coccolithophore blooms when using remote sensing (because foraminifera or pteropods are typically much less abundant and scatter so little light per unit mass of PIC). On the other hand, the mass-specific backscattering coefficient for calcite is not constant for the various species of coccolithophores (Balch et al. 1999) due to differences in coccolith morphology and degree of platedness. This can cause biases in the blooms that are observed by satellites since species with small coccoliths are more likely to be visible optically. It also sets the likely lower limit for the accuracy of any remote sensing calcite algorithm.

With the above caveats in mind, several remote sensing approaches have been applied to the study of coccolithophores, mostly *E. huxleyi*. The Coastal Zone Color Scanner (CZCS) was used early-on for *E. huxleyi* bloom observations, and differences were noted in the remote sensing reflectance spectra of coccolithophorids compared to other algal assemblages (Holligan et al. 1983). The Advanced Very High Resolution Radiometer (AVHRR) placed on various NOAA satellite platforms offered valuable information on coccolithophores, especially after the demise of CZCS. The AVHRR approach was to use the broad-band, but low sensitivity, channel 1 and correct it for clouds by subtracting the infra-red channel 2 (Groom and Holligan 1987). The approach allowed definition of bloom boundaries, but was not highly quantitative in terms of the amount of PIC or coccolith abundance, due to inaccuracies in the estimated radiances. Nonetheless, the AVHRR approach allowed important documentation of blooms after the demise of CZCS and before the launch of the follow-on Ocean Color Thermal Sensor (OCTS) and Sea Wide Field-of-view Sensor (SeaWiFS).

Another approach, used mostly with SeaWiFS, was to generate coccolith flags, (i.e. regions within images with a high probability of being dominated by coccolithophores). These flags have been invaluable for indicating regions where the chlorophyll algorithm would likely fail (Brown and Yoder 1994a). Ackleson et al. (1994) also derived a technique for relating suspended calcite to radiance, based on Gulf of Maine data. Gordon et al. (1988) derived a quantitative, two-band algorithm for deriving the concentration of PIC, based on absolute values of 440 and 550 nm water-leaving radiance, not radiance ratios. Their algorithm required, as input, the calcite-specific backscattering coefficient for *E. huxleyi* (Balch et al. 1996a, 1996b, 1999). The two-band algorithm iteratively solved for calcite concentration and chlorophyll and is currently being implemented by the Moderate Resolution Imaging Spectroradiometer (MODIS) sensor aboard NASA's Terra and Aqua satellite platforms (Esaias et al. 1998). A three-band calcite algorithm has also been derived, based on red and near infra-red bands (Gordon et al. 2001).

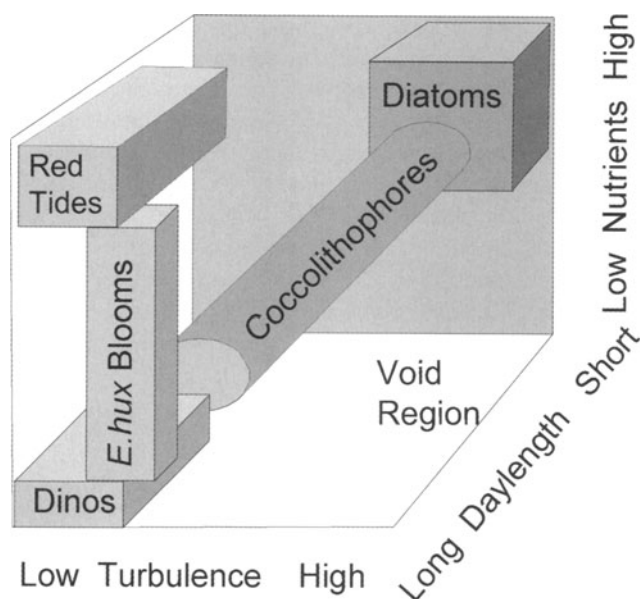


Fig. 9. Three-dimensional phytoplankton mandala in which a daylength axis has been added to Margalef's original two-dimensional mandala of nutrients and turbulence (Margalef 1978). Also *E. huxleyi* blooms have been added. Here they are shown to occur in moderately low turbulence, but not the lowest turbulence waters characteristic of dinoflagellate populations. This is based on numerous field observations of blooms forming on the moderately-stratified side of frontal boundaries. The ecological response of *E. huxleyi* is separated from that of coccolithophores in this diagram since its physiological ecology is not typical of all coccolithophore species (Young 1994).

The benefit of this latter algorithm is that it is not subject to absorption effects of chlorophyll and colored dissolved organic matter. This algorithm currently is being implemented with SeaWiFS data.

The two-band PIC algorithm of Gordon et al. (1988) has been validated, mostly using our data from the Gulf of Maine. Results suggest that the maximum RMS error of the algorithm is $\pm 15 \mu\text{g PIC l}^{-1}$. This is about 5–10% of the PIC concentrations observed in a dense coccolithophore bloom but still greater than most PIC concentrations in non-bloom situations. One of the reasons for this error value is the wide variety of PIC particles in the sea (e.g., the error analysis includes regions of suspended calcite sediments). For example, areas over Georges Bank frequently show high PIC concentrations but this likely is because the algorithm is “fooled” by high suspended sediments (in this case, sands). Nonetheless, in re-

gions where there are no high concentrations of suspended sediments or other minerals, and the range in size of the PIC particles is fairly uniform, the precision of the two-band algorithm is $\sim 3\text{--}5 \mu\text{g PIC l}^{-1}$, within the range of ambient PIC concentrations (details to be published separately). Moreover, by time and space binning of satellite PIC data, the standard error of the remote PIC estimates can be reduced sufficiently to allow PIC determination in the oligotrophic central oceans.

The first global views of surface PIC are being produced by SeaWiFS and MODIS and the results are striking (Fig. 10). Seasonal images clearly illustrate the importance of day length on the appearance of high PIC concentrations (as mentioned earlier). That is, PIC concentrations in northern and southern hemisphere are maximal close to the respective summer solstice. Nowhere is this more obvious than in the North Atlantic and Argentine Continental Shelf regions. As described earlier, both are regions of known coccolithophore blooms (Holligan et al. 1993). Another region that shows enhanced PIC concentrations is the Polar Frontal region of the Southern Ocean during the Austral summer. This is not an area known for coccolithophore blooms, and these results must be interpreted cautiously until more sea-truth data are available. This cautionary note is especially relevant considering that the Polar Front region is better known for diatom populations (Brzezinski et al. 2001) and, if biogenic silica is in high enough concentration, its backscattering might be confused with that of calcite. Nonetheless, the two-band algorithm was derived originally based on the light scattering properties of mixed phytoplankton assemblages, including diatoms. Thus, it should compensate for diatom scattering, as long as that scattering is associated with living cells, not suspended frustules (containing no chlorophyll). It is also worth noting that the satellite view of PIC distributions are biased to coccolithophores inhabiting surface waters (that is, the top optical depth of the water column (Gordon and McCluney 1975)). Thus, deeper coccolithophore populations (with different ecological strategies) would not necessarily be detected.

Can large-scale PIC distributions be related to Margalef's Mandala?

Given global maps of coccolithophore PIC, it is tempting to conclude that regions of high PIC are regions of high coccolithophore abundance (and that one can use carbon and cell abundance interchangeably within Margalef's Mandala). This interpretation is dangerous, however. For example, in the Arabian Sea, the highest coccolithophorid abundance was during the intermonsoon period, yet the highest calcification rates were during the Monsoon, the most productive period (Balch et al. 2000, 2001). In the Gulf of Maine, the highest calcification rates were not necessarily in regions with the most suspended PIC but often where overall primary production was greatest. In non-bloom situations, the typical calcification rate by the entire phytoplankton assemblage is between $0.1\text{--}30 \text{ g C g Chl d}^{-1}$ (low, when compared to typical assimilation ratios for phytoplankton of $20\text{--}200 \text{ g C g Chl d}^{-1}$

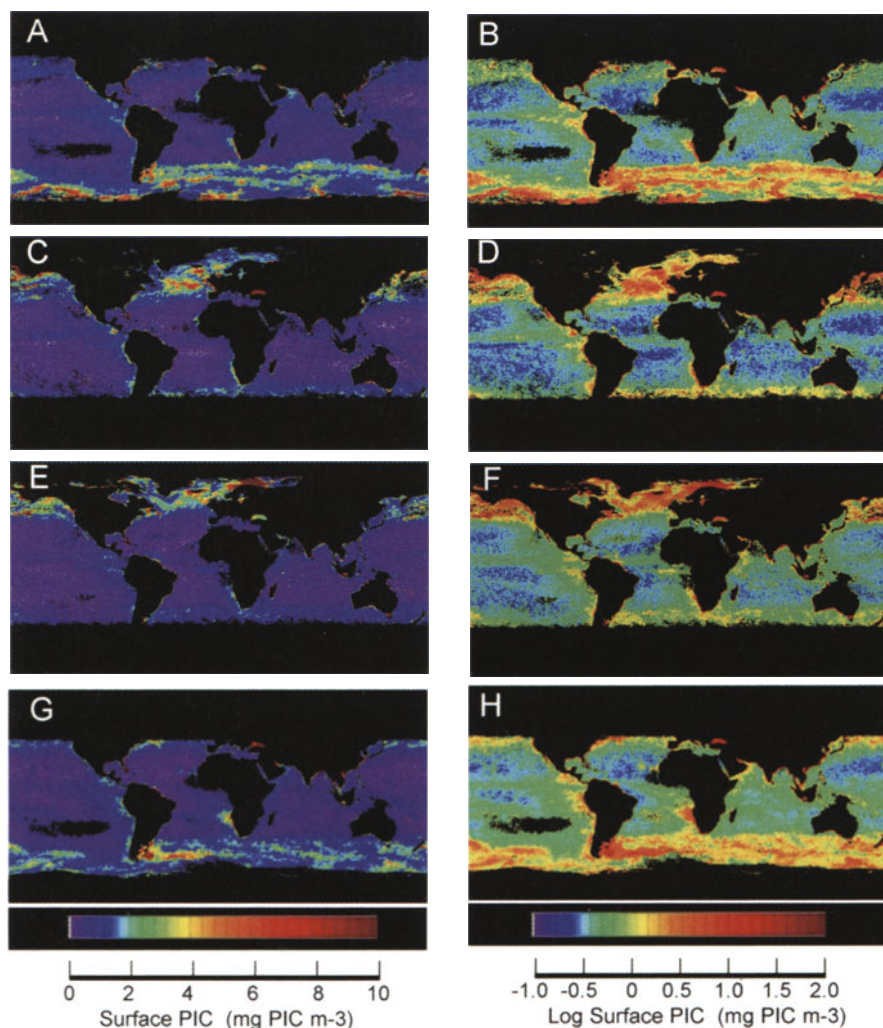


Fig. 10. Global views of PIC concentration taken by MODIS-Terra. Images were produced using Version 4 software, by performing averages of monthly mean images. Color scales on left-hand plots are linear, ranging from 0 (indigo) to 10 mg PIC m⁻³ (red), those on right-hand plots are logarithmic scales, with the following color scale in units of mg PIC m⁻³: 0.1=indigo, 1=green, 10=orange, 100=red. Seasonal periods are as follows: **A.**; **B.** January through March, **C.**; **D.** April to June, **E.**; **F.** July to September, and **G.**; **H.** October to December. PIC concentration was calculated using the two-band PIC algorithm based on Gordon et al. (1988).

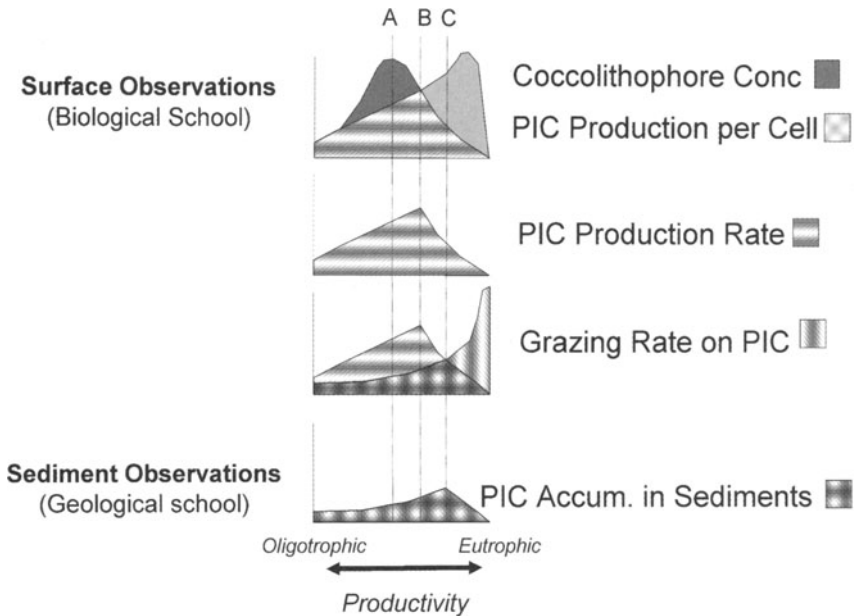


Fig. 11. Conceptual model for how coccolithophores can be most abundant in mesotrophic surface waters (“biological school”), yet they accumulate fastest in sediments underlying more eutrophic regions (“geological school”). Coccolithophore abundance has been shown along a gradient of productivity. Coccolithophore abundance, as suggested from the Margalef mandala, should peak in mesotrophic regions (line A). The product of the two curves for coccolithophore concentration and the calcification per cell (see Fig. 4), represents the total PIC production rate (second plot down). The curve in the PIC production rate (line B) is biased towards the higher production regions. Multiplying the PIC production curve by a variable PIC grazing rate (which is even more biased towards higher production regions) yields a PIC accumulation rate in the sediments that is highly biased towards high productivity zones (line C). Note, no aspects of carbonate preservation/ dissolution are incorporated into this simplified view, only aspects of surface PIC production and grazing.

in the Gulf of Maine). The result is that calcification/photosynthesis ratios are not constant and, not surprisingly, vary from 0.1% to values >30%.

Studies of coccolithophore C fixation versus cell abundance have resulted in two fundamentally different schools of thought concerning coccolithophore distributions in space and time. The “geological school” associates sediments containing high numbers of coccolithophores with mixed overlying waters of high productivity. This is entirely consistent with our Arabian Sea and Equatorial Pacific carbon fixation observations. The Margalef Mandala, on the other hand, represents the “biological school”, in which coccolithophores are more likely found in moderately stratified waters, of moderate to low productivity. The many observa-

tions of healthy coccolithophore populations living on the stratified sides of frontal boundaries, next to well-mixed waters containing diatoms, supports the biological school. Both views can be considered internally consistent given that the calcification rate per cell is extremely variable (as shown in Fig. 4). This would be expected for different coccolithophore species, with different ecological strategies. This, combined with differences in grazing, can be incorporated into a conceptual model and used to explain the biological and geological views of coccolithophore abundance (Fig. 11). That is, the build-up of coccolithophore biomass in moderately stratified waters results from calcification occurring in an environment with 1) few grazers (which otherwise accelerate the downward flux of the PIC to the underlying sediments as ballasted fecal pellets) and 2) minimal vertical mixing. In highly productive situations, large grazers are more likely to be present, such that coccoliths and coccospheres would be rapidly transported to the sediments in fecal pellets and preserved in the geological record. Peak calcification would be observed in high productivity scenarios, as well, since populations are growing rapidly. In situations of moderate stratification, grazer abundance is more stochastic, such that there is a potential for a build-up of ungrazed coccolithophore PIC. Low grazing and high water column stability (low vertical mixing) mean that slow-sinking coccoliths can build-up to sufficient concentrations to cause turbid "blooms". One important caution, however, is that grazing can also be associated with increased PIC dissolution (Harris 1994; Milliman et al. 1999); unfortunately, insufficient data exist to incorporate this into the conceptual model. Nonetheless, this conceptual model illustrates why interpreting the Margalef Mandala in terms of carbon does not necessarily follow from a conceptual model based on only cell abundance. Future efforts to understand coccolithophore abundance in space and time (from biological to geological time-scales) will require better knowledge of the PIC transfer functions between the water column and sediments.

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Structure and morphogenesis of the coccoliths of the CODENET species

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Summary

Detailed descriptions are given of the structure and morphology of the heterococcoliths of the six CODENET taxa; *Gephyrocapsa oceanica*, *Coccolithus pelagicus*, *Calcidiscus leptoporus*, *Umbilicosphaera foliosa*, *Helicosphaera carteri* and *Syracosphaera pulchra*. These descriptions highlight the quality of phylogenetic data which can be obtained from coccoliths, and the coccoliths of the closely related genera *Coccolithus*, *Calcidiscus* and *Umbilicosphaera* are compared to explore the interplay between evolutionarily conservative and non-conservative aspects of morphology. These case studies emphasize that identifying homologies between coccoliths is most meaningfully done at the crystal unit level rather than the overall morphology level. This way, focus is kept as close as possible to the process of biomineralisation itself, which is essential because a minor shift in process can cause a large morphological change – exemplified here particularly by development of bicyclicity in shield elements.

These case studies are also used to develop our understanding of coccolith biomineralisation processes. In particular: (1) The role of crystal growth in controlling aspects of coccolith structure is highlighted. (2) The unique morphology of *H. carteri* coccoliths is related to a growth model. (3) The lath elements of *S. pulchra* are shown to have tangential c-axis orientations, resulting in an important modification of the V-/R-model.

Introduction

Coccoliths are remarkable biomineral structures. They are formed by unicellular algae and borne extracellularly, yet they show extraordinarily elaborate form and

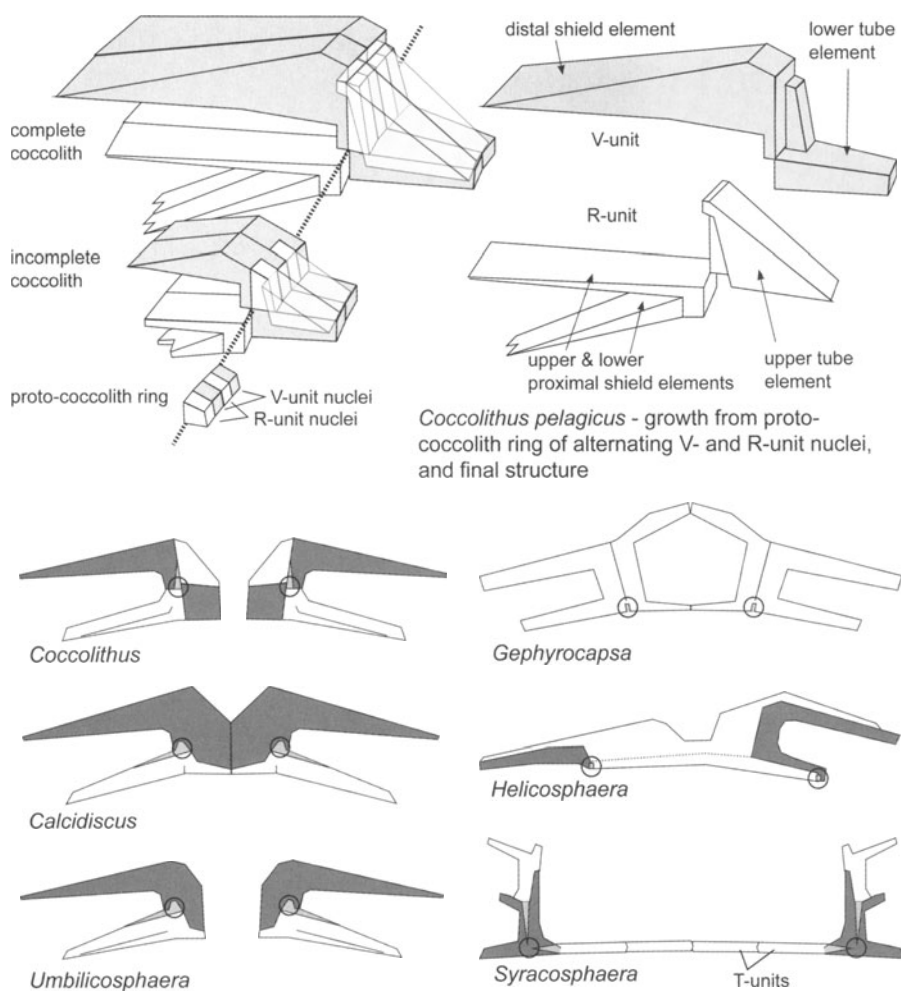


Fig. 1. V-R model interpretation of *Coccolithus pelagicus* structure and comparative cross-sections of the six genera. Conventions in lower diagrams: white – R-units; dark gray – V-units; circles – locus of proto-coccolith ring.

very precise regulation of crystal nucleation, orientation and growth. As a result coccolith biomineralisation has attracted considerable interest as a model system for understanding the processes by which organic systems can control inorganic crystal growth. To understand the biochemical systems involved, two species, *Emiliania huxleyi* and *Pleurochrysis carterae* have been widely used as case studies (e.g. de Vrind de Jong et al. 1997; Marsh 2000). In addition their morphology has been extensively investigated because biologists, and especially paleontologists, depend upon these products of the biomineralisation process for identifi-

cation and classification of coccolithophores. Convergence of these approaches has shown that heterococcoliths are formed by a growth process commencing with nucleation of a simple proto-coccolith ring of oriented calcite crystals on an organic baseplate scale (Klaveness 1976; Young 1989), followed by regulated growth of these crystals. Typically the proto-coccolith ring consists of calcite crystals with alternately sub-vertical and sub-radial c-axis orientations; V- and R-units (Young and Bown 1991; Young et al. 1992, 1999; Marsh 1999). This pattern is illustrated in Fig. 1, the schematic drawings of two segments of *Coccolithus pelagicus* rim (redrawn from Young 1993) show how the complex final form develops from an initial ring of alternating V- and R-unit nuclei. Recognition of this pattern has formed the basis for revision of coccolith classification and evolution (e.g. Bown and Young 1997; Young and Bown 1997).

This paper describes the structures of the heterococcoliths produced by taxa selected for the CODENET project; *Coccolithus pelagicus*, *Calcidiscus leptoporus*, *Umbilicosphaera foliosa*, *Helicosphaera carteri*, *Gephyrocapsa* and *Syracosphaera pulchra*. The basic structures of these species are reasonably well-established (Fig. 1) but the information is scattered across the specialist literature. The purpose of this paper is to provide a set of case studies from across extant coccolithophorid phylogeny to illustrate the diversity of heterococcolith biomineral structures produced. The set of studies also provides for biologists, and others interested in coccolithophorid phylogeny, an insight into the nature of phylogenetic data which is provided by coccolith ultrastructure. The CODENET species selection was designed to maximize sampling of large scale diversity from a limited set of species and so is ideal for our purpose. In addition the set of case studies allows a series of issues on coccolith biomineralisation to be addressed. In particular we focus on element morphogenesis, in all species; on the development of the chiral flange structure in *Helicosphaera*; and on documentation of a very distinctive nucleation mode in *Syracosphaera pulchra*. Implications of these results for coccolith biomineralisation are discussed.

Material and Methods

The main material used for this study consisted of culture samples of coccoliths. Culture methods are described by Probert and Houdan (this volume). To obtain well-formed coccoliths sampling during the exponential or early stationery growth phase was used. Representative culture samples with well-formed coccoliths were studied intensively in the SEM with numerous coccoliths imaged. Oceanic filter samples were also used, but culture samples are particularly useful since very large numbers of specimens are available including coccoliths at varying growth stages, and there are no problems of taxonomic identification. SEM observations were made using a Phillips XL30 FEG, with digital image capture.

Complementary observations were made using cross-polarized light microscopy (LM) to determine broad crystallographic orientation (see e.g. Young 1993;

Moshkovitz and Osmond 1989), or to verify this since for most species it has been determined previously. Light microscope (LM) observations were made using a Zeiss Axioplan photomicroscope, again with image capture facilities.

Atomic force microscopy (AFM) was also applied to some of the species (Henriksen et al. 2003, in press) providing key evidence that rhombohedral faces are widely developed, highlighting particular aspects of the morphology of some species, and providing new data on organic phases. Observations given here are, however, all either based on or supported by scanning electron microscopy.

Examination of high resolution AFM and SEM images has made it increasingly clear that most surfaces are either biologically shaped non-crystallographic faces or calcite rhombohedral faces (Henriksen et al. in press). The rhombohedral faces are characterized by flat surfaces, rhombic shape and the parallelism of edges. Where rhombohedral faces appear to be developed it is usually possible to infer crystallographic orientation. A calcite rhombohedron can be likened to a slightly flattened cube (Fig. 2). The principal axis of symmetry is the c-axis, a three-fold rotational axis (or six-fold axis of rotation-inversion symmetry). All six rhombic faces of a calcite rhombohedron are identical, they have two acute angles (78°) and two obtuse angles (102°) and the c-axis bisects the obtuse angle. The interfacial angles along the radial edges are 106° whilst the interfacial angles along the equatorial edges are 74° . These relationships can be used to determine the crystallographic orientation of rhombohedral crystals provided at least two faces are visible. Since the broad crystallographic orientation is also available from light microscopy observations combining data from these approaches allows analysis of coccolith crystallography.

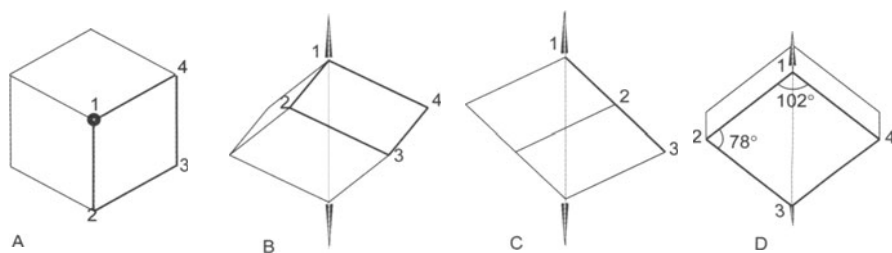


Fig. 2. Four views of a calcite rhombohedron with a single face highlighted and its vertices numbered. C-axis is represented by conical symbols. Views are respectively oblique, parallel and perpendicular to the highlighted face. The face perpendicular view is frequently the most useful for interpreting images.

Results

Gephyrocapsa

Gephyrocapsa coccoliths are similar in structure to those of *Emiliania huxleyi*, the best studied and described single species (Westbroek et al. 1989; Young 1994; Didymus et al. 1994; Davis et al. 1995). Indeed all coccoliths of the family Noelaerhabdaceae have essentially similar structure, differing only in relatively minor details such as presence of slitting between elements and development of additional central area structures (Young 1989). Coccolith growth starts from a proto-coccolith ring of alternating V- and R-units, but only the R-units are developed (Young et al. 1992). These grow outward to form the proximal shield, inward to form the grill and upward in two directions to form inner and outer tubes (Fig. 1). The distal shield then develops from the outer tube. The bridge is an additional structure developed by selective growth of a few elements of the inner tube.

High resolution images of *Gephyrocapsa* are shown in Fig. 3. There are few obvious flat crystal faces. The distal surfaces of the shield elements are very regular and often have a ridge parallel to their length. They are not rhombohedral faces since the c-axis lies in the plane of the shield (Davis et al. 1995; our LM obs.), so all potential rhombohedron faces are steeply inclined relative to it. Our observations suggest that the ends of the bridge elements (e.g. Fig. 3A) and at the tips of shield elements (Fig. 3B, C) are rhombic faces. As the shield elements grow radially outward and the bridge inward, growth must be concentrated on these edges, suggesting an association between crystal face development and growth fronts. In *G. oceanica* (Fig. 3A), and *E. huxleyi*, the rhombohedral facets are often not present on complete coccoliths but are seen at intermediate growth stages.

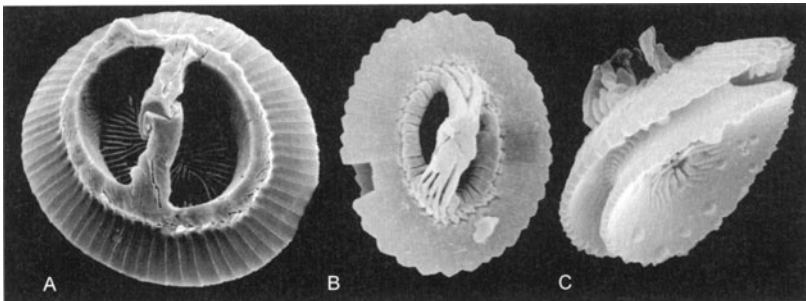


Fig. 3. *Gephyrocapsa* coccoliths: **A.** *G. oceanica* in distal view. **B.** *G. muelleriae* in distal view. Note rhombohedral facets on ray tips of both distal and proximal shields. **C.** *G. muelleriae* in oblique view.

Coccolithus pelagicus*, *Calcidiscus leptoporus* and *Umbilicosphaera foliosa

These three species are closely related, as inferred first from coccolith structure (e.g. Perch-Nielsen 1985; Young 1989) and supported by molecular genetics (Saez et al. this volume). Hence it is convenient to consider them together. The structure of each species is briefly outlined below and shown in cross-section in Fig. 1. Then various features common to the three species are discussed in more detail, and illustrated in Figs. 4–6.

Coccolithus pelagicus

The heterococcoliths of *Coccolithus pelagicus* have a relatively complex structure, as described in detail by Young (1994) and Henriksen et al. (2003, in press). Unlike in *Gephyrocapsa* and the other reticulofenestrid coccoliths, both V- and R-units are well developed. The V-units form the distal shield, which consequently is dark in plan view in cross-polarized light. Less obviously they also form the lower layer of the central area (Fig. 1, Young 1994). The R-units form the proximal shield, which consequently is bright in plan view in cross-polarized light. The units grow out to form a shield of two layers, an upper one of radially directed elements and a lower one of oblique elements. This scissor-structure is analogous to the wall layers of *Emiliana* with the two elements being formed from the same crystal-units. The R-units also form the upper layer of the central area which consequently is also bright in plan view in cross-polarized light, although with careful focusing it is evident that this birefringence is confined to the upper layer (Young 1994). The two units interlock around a belt within the coccolith structure, which is only exposed on broken specimens. This belt of alternating V- and R-units is the locus of the proto-coccolith ring and thus the position of nucleation of the crystal units. The nucleation probably occurs on the baseplate scale which becomes embedded in the structure during subsequent growth (Henriksen et al. in press).

In addition *C. pelagicus* possesses a central bar that is formed by separate nucleation.

Calcidiscus leptoporus

The basic *Calcidiscus* structure was worked out by Kamptner (1954) and Gartner (1967) and reinterpreted in terms of the V/R model by Young et al. (1992) and, more accurately, by Kleijne (1993). The structure is similar to that of *Coccolithus* in that the distal shield is formed of V-units and the proximal shield of R-units and the proto-coccolith ring locus is embedded within the tube and therefore only visible on broken specimens (Fig. 1). It differs in having the shield elements extending directly into the central area without a cross-over zone. Thus, the upper part of central area is formed from V-units and the lower part from R-units. This results in a simpler appearance than *C. pelagicus* in cross-polarized light. It also means that the two shields are only weakly connected and often separate.

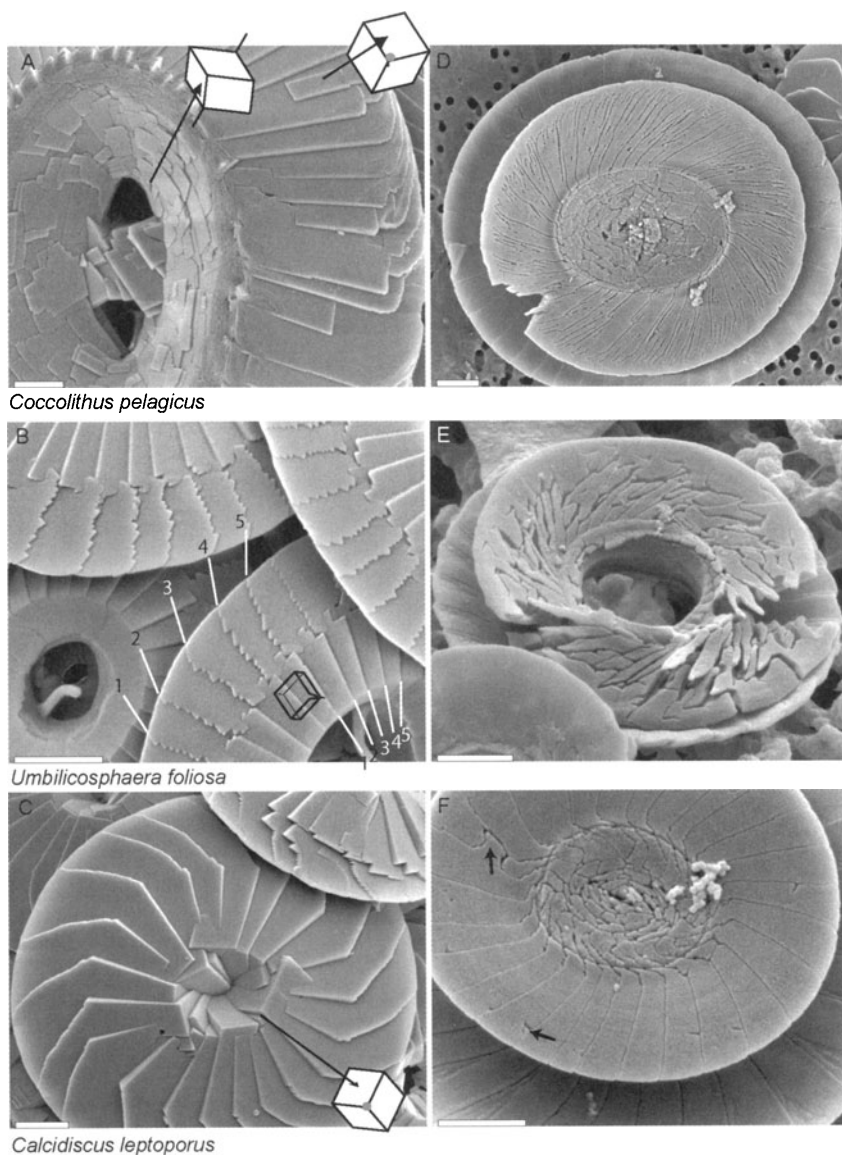


Fig. 4. *C. pelagicus*, *U. foliosa* and *C. leptoporus* coccoliths. Scale bars 1mm. **A–C**, distal views. Virtually all surfaces are rhombohedral calcite faces, rhomb symbols indicate crystallographic orientation. In *U. foliosa* (B) numbers indicate parallel edges on offset parts of single crystal units (see also Fig. 5). **D–F** proximal views. All surfaces are non-crystallographic, as indicated by curvature of surfaces and absence of straight edges. Proximal shield in *C. pelagicus* and *U. foliosa* is bicyclic, this is not shown in *C. leptoporus* but clockwise directed extensions of a few elements (arrows) are probably analogous.

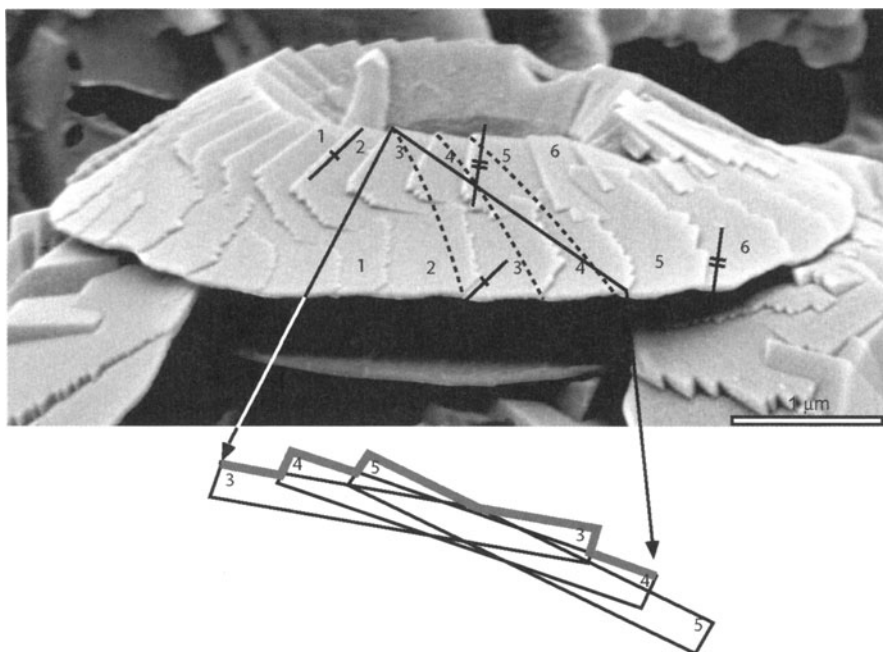


Fig. 5. Side view of an *Umbilicosphaera foliosa* coccolith showing the bicyclicity. Numbering on the crystal units indicates the degree of offset between the inner and outer cycles. The continuity of the crystal units between the cycles is directly visible on units 1 and 2, and is further indicated by development of parallel edges on the inner and outer cycles (lines on leading and trailing edges of units 2 and 5). Dotted lines indicate summary trajectory of units 3 and 4, this is approximately the pattern seen on the proximal surface of the distal shield. Diagram below; oblique section across the coccolith surface illustrating how the differing senses of apparent imbrication in the inner and outer cycles can be produced by a single set of crystal faces.

Umbilicosphaera sibogae* and *foliosa

The structure of *Umbilicosphaera* is essentially the same as that of *Calcidiscus*. V-units form the distal shield and upper part of the tube, while R-units form the proximal shield including the lower part of the tube (Fig. 1). Indeed the two genera are only differentiated by the relatively unconvincing characters that there is a wide central opening in *Umbilicosphaera* and that the distal shield sutures are more complex in *Umbilicosphaera*. Differences between *U. foliosa* and *U. sibogae* are described in Geisen et al. (this vol.) and only *U. foliosa* is illustrated here.

Distal shield elements

The suture patterns on these three genera are variable (Fig. 4A–C). The sutures on *Coccolithus* are essentially straight, whereas they are curved on *Calcidiscus*. They are more complex on *Umbilicosphaera*, with a division between an inner part showing clockwise imbrication and an outer part showing anti-clockwise imbrication. However, with high resolution SEM images it is evident that the main surfaces of the distal shield elements of all three genera are flat, probably crystallographic, faces, bounded by angular edges. AFM has proven that they are rhombic crystal faces in *C. pelagicus* (Henriksen et al. 2003, in press), strongly suggesting that they are in the other species as well.

Whereas the typical *Calcidiscus* and *U. foliosa* distal shields appear very different (monocyclic vs. bicyclic) intermediates can be seen, notably on *U. sibogae* and on the “small” *Calcidiscus* sub-species (Quinn et al. this volume; Geisen et al. this volume). From examination of good SEMs it is evident that the inner and outer cycles on the *U. foliosa* distal shields are formed from the same crystal units and indeed are both formed from the same rhombohedral faces (Figs. 4B, 5). The difference in apparent imbrication is a product of the obliquity of the crystal units and curvature of the coccolith surface resulting in differential growth of the clockwise directed edges of crystals in the inner part of the coccolith and anti-clockwise directed edges on the outer part of the coccolith, as shown in Fig. 5.

The lower surface of the distal shield in all three genera has a much simpler appearance (Fig. 6A–C) with nearly straight, slightly oblique sutures and a smooth surface without crystal face development.

Proximal shield elements

The proximal shields show variation in complexity comparable to that of the distal shields. In *Calcidiscus* (Fig. 4F), and *U. sibogae* (Geisen et al. this volume), the proximal shield is relatively simple being formed of a single cycle of radial elements. By contrast the shield is bicyclic in *U. foliosa* and *Coccolithus* (Fig 4D–E, 6A). It is clear that the bicyclic structures of *U. foliosa* and *Coccolithus* are analogous. Common features of this structure in the two species include: clockwise obliquity of the lower elements in proximal view; extension of the upper layer beyond the lower layer, and complex terminations of the lower layer elements. However, since the Miocene *Umbilicosphaera* species (*U. jafari* and *U. rotula*) are monocyclic, it is very difficult to see how the features could have a common phylogenetic origin, i.e. be directly homologous. Moreover similar bicyclic proximal shields also occur in some Miocene *Calcidiscus* specimens (Janin 1992), and in *Oolithotus antillarum*, but not in *O. fragilis* (our obs.). These species are distributed across the phylogeny of the Calcidiscaceae so a single phylogenetic origin really is not possible. Further evidence for this being an intrinsic crystallography-related tendency is the common presence of clockwise directed extensions of proximal shield elements in *C. leptoporus* (Fig. 4F) and in *O. fragilis*.

A possible pattern is that the bicyclic shields develop when the proximal shield is relatively thick and wedge shaped, and are absent on thin and parallel-sided

proximal shields, this contrast is particularly suggestive for the *U. sibogae* vs. *U. foliosa* contrast, but it is also broadly paralleled in the cases of *Coccolithus pelagicus* vs. *Calcidiscus leptoporus* and of *O. fragilis* vs. *O. antillarum*. So a possible explanation for the presence of the feature in only some species is that the basic structure and crystal arrangement of the shield allow the development of two layers, but only if significant downward growth from the proto-coccolith ring occurs prior to lateral growth of the shield. This might allow separate growth directions to develop on the upper and lower surfaces. The upper, near-radial growth direction is parallel to the c-axis so a possible explanation of the lower, oblique growth direction is extension perpendicular to a rhombohedral calcite face.

The upper surface of the proximal shield is much more uniform between the species, with straight sutures showing weak clockwise obliquity (Fig. 6D–F). A distinctive feature of this surface in *Calcidiscus*, *Umbilicosphaera* and some *C. pelagicus* specimens is the presence of lunate facets around the central opening (Fig. 6D–F). In *Umbilicosphaera* small extensions of the V-units can be seen on isolated distal shields (Plate 6B) and must form part of the top of the proximal shield and so fill the lunate facets. These V-unit extensions onto the proximal shield have not been directly observed in *Calcidiscus* or *Coccolithus*, probably because they are delicate and easily lost during shield separation, but the lunate facets provide strong evidence that they are present. This reinforces the marked uniformity of the upper surfaces of the proximal shield in these genera.

Tube elements

The tube elements are apparently very different in the three species. The upper tube elements are a mass of R-unit derived elements in *C. pelagicus* (Fig. 4A) but relatively regular V-unit derived elements in *Calcidiscus* and *Umbilicosphaera* (Fig. 4B–C). The lower tube elements appear on the proximal surface as irregular V-unit derived elements in *Coccolithus* (Fig. 4D), as irregular R-unit derived elements in *Calcidiscus* (Fig. 4F) and as a narrow belt of regular R-unit elements in *Umbilicosphaera* (Fig. 4E). However, on the isolated shields (Fig. 6) more homologous features are evident. On the isolated distal shields of *Umbilicosphaera* and *Calcidiscus* the upper tube elements (formed from V-units) both show clockwise imbrication and this can also be seen in the lower tube elements of *C. pelagicus* (also formed from V-units). Similarly the isolated lower tube elements in *Umbilicosphaera* and *Calcidiscus* both show anticlockwise obliquity.

In conclusion it seems that in these genera the concealed surfaces of the shields, which are directly derived from the proto-coccolith ring, show much greater similarity than the exposed surfaces, which are formed by growth further from the proto-coccolith ring.

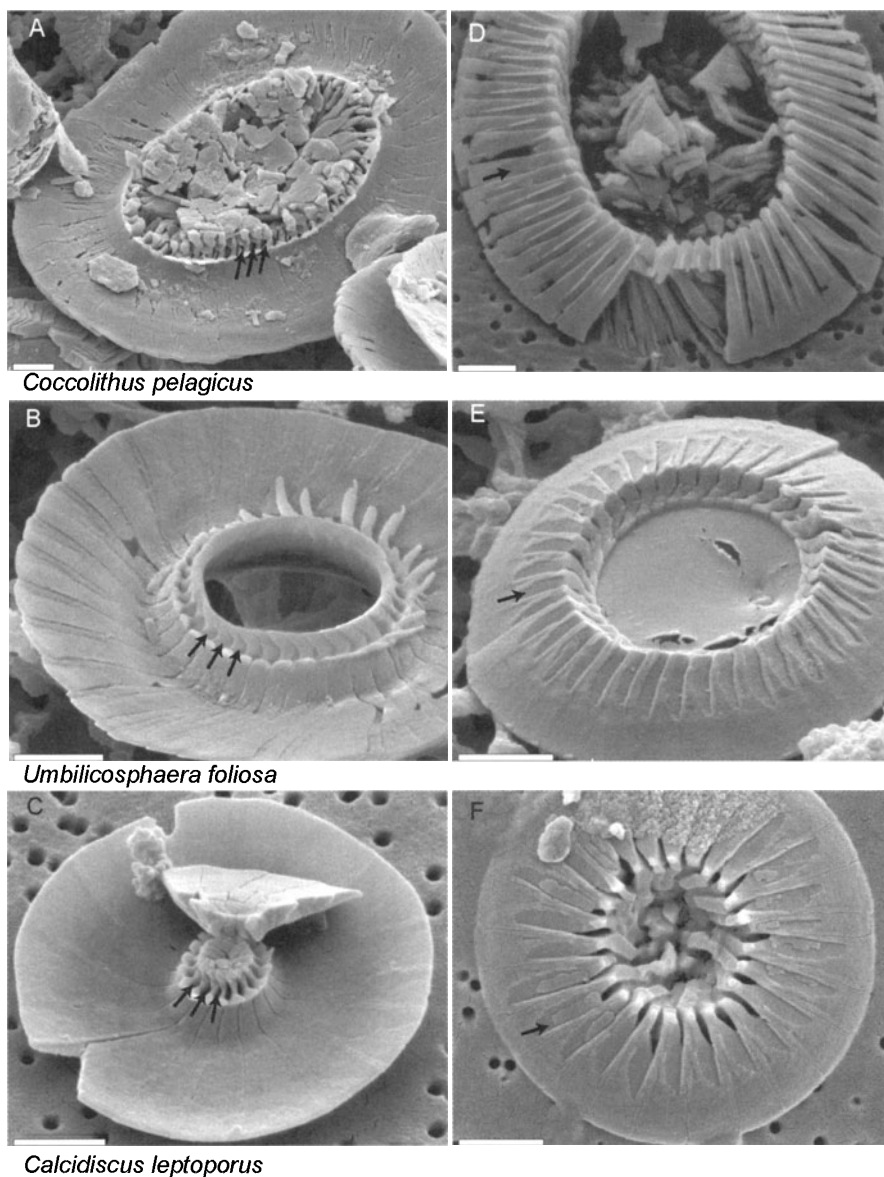


Fig. 6. *C. pelagicus*, *U. foliosa* and *C. leptoporus* coccoliths. Scale bars 1 mm. **A–C:** Isolated distal shields seen in proximal view, more precisely these are isolated V-units, so in the case of *C. pelagicus* the lower tube cycle is present (compare with Fig. 1). Arrows – holes marking location of R-unit nuclei on proto-coccolith ring. **D–F:** Isolated proximal shields seen in distal view, these are entirely formed of R-units (although in the case of *C. pelagicus* the extensions of the R-units onto the distal surface are absent). Arrows – lunate facets marking V-unit extensions onto the proximal shield.

Helicosphaera carteri

Helicosphaera coccoliths have a unique morphology with a spiral flange and have attracted much interest, with detailed descriptions by Kamptner (1954), Black and Barnes (1961), Clochiatti (1969), Theodoridis (1984), and Aubry (1990).

The basic morphology and terminology of *Helicosphaera* coccoliths is illustrated in Fig. 7. Henriksen et al. (in press) show that the spiral flange is formed of V-units whilst the proximal plate and blanket are formed of R-units. These originate in a proto-coccolith ring that is clearly exposed on the proximal surface, around the edge of the proximal plate (Fig. 8B). This is the most elegant and readily observed proto-coccolith ring locus of any extant coccolithophore.

The R-unit elements of the blanket and proximal plate have radically different appearances. The proximal plate elements are large discrete elements, grown radially inward from the proto-coccolith ring, and they normally have rather level non-crystalline surfaces (although occasionally with small facets, Fig. 8B). By contrast, the blanket is formed of numerous minute rhombohedral crystal faces dominated by tangentially aligned edges (Fig. 8D–F). As shown by Henriksen et al. (in press) these minute faces are not separate crystals but steps on larger crystal units. In the central area no rhombohedral face is parallel to the coccolith surface, so the steps are closely-spaced and appear needle-like (Fig. 9M–O). On the distal surface, where the blanket overlaps the flange, a rhombohedral face is sub-parallel to the coccolith surface and larger rhombic faces develop (Fig. 9O).

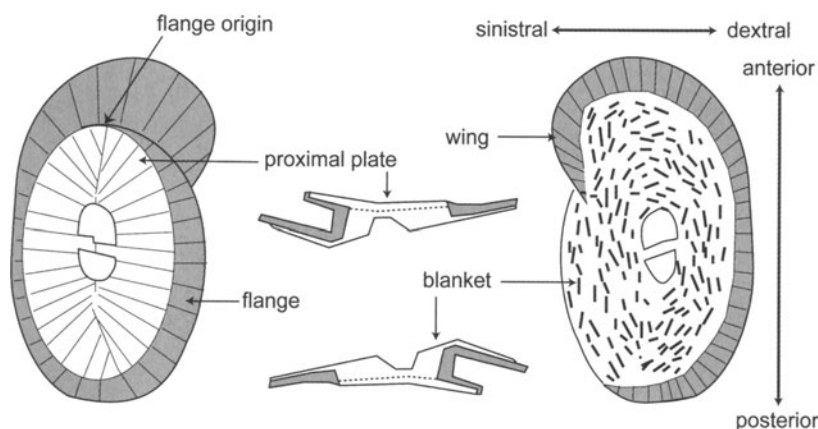


Fig. 7. *Helicosphaera* structure and terminology, redrawn and modified from Young et al. (1997). Proximal view, cross-section and distal view. N.B. The cross-section is shown up-side-down as well as in normal orientation in order to make its relationship to the proximal view clearer.

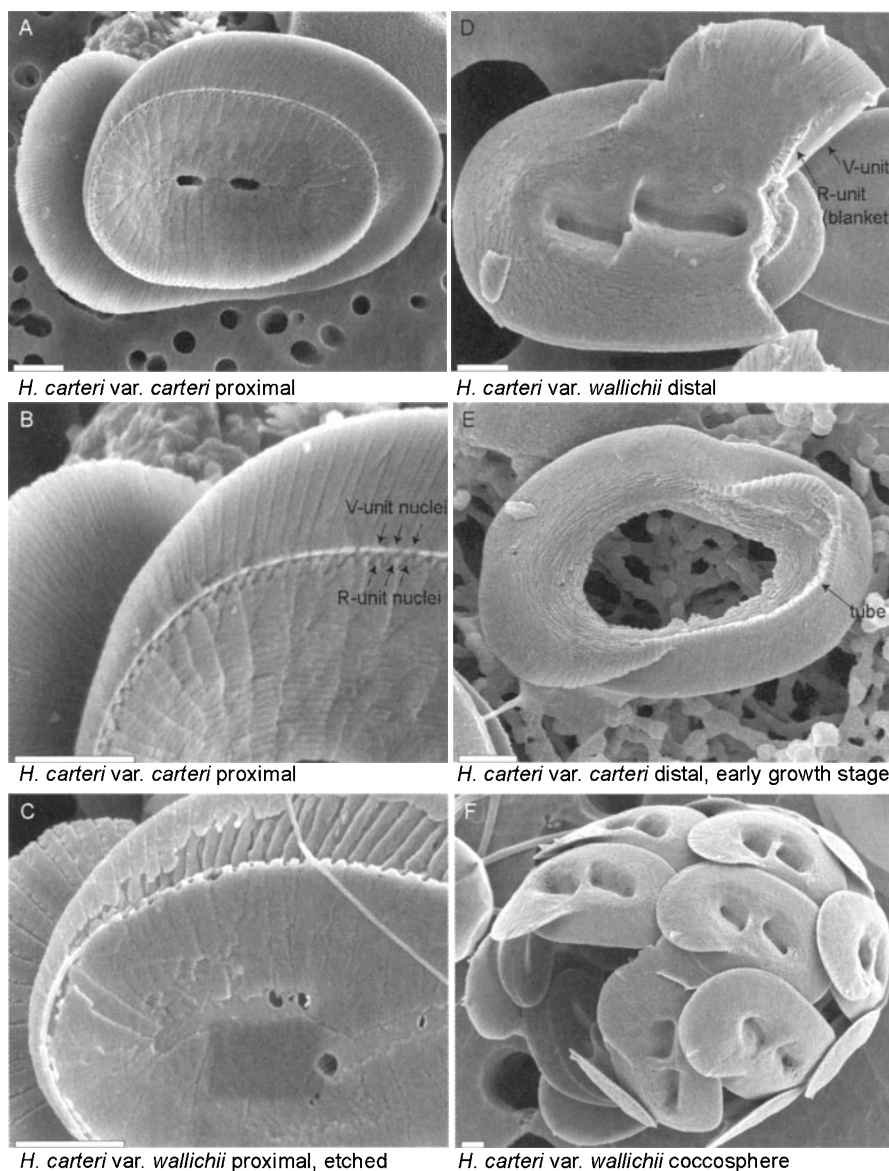


Fig. 8. *Helicosphaera* morphology. Scale bars one micron. **A–C:** Proximal views. On the etched specimen (C), V-units of base of flange partially removed, revealing the overlying blanket (compare with profile in Fig. 7). **D–F:** Distal views. The incompletely grown specimen (F) is in same orientation as specimen above; note contrast in appearance between antero-dextral corner (bottom right) with fine oblique sutures and postero-sinistral corner (top left) with irregular smooth appearance.

The V-unit elements are mostly concealed by the blanket elements on the distal surface (Fig. 8D) but show crystal facets where exposed. At the end of the wing they often develop horn-like protrusions (Fig. 8D, F), the front end of which is probably a rhombic face (Fig. 8F). On the proximal side they show finely striated non-crystallographic surfaces.

Growth

In natural populations *Helicosphaera* coccoliths at early growth stages are rare, but in cultures they are normally present and often very common. They are typically characterized by open central areas and weak or abnormal wing development (Fig. 8E). Assembling a large number of micrographs of these coccoliths has allowed the growth sequence to be worked out for the first time (Fig. 9). A necessary preliminary step in doing this was to orientate the specimens. With complete *Helicosphaera* coccoliths it is convenient to orientate specimens with the long axis vertical and the wing toward the top. In incomplete specimens, however, the wing is absent and so it is more difficult to distinguish the anterior and posterior ends. In proximal views the flange origin provides a unique reference point, at the anterior end (Fig. 7). In distal views highly variable flange development makes identifying homology difficult. However, there is a useful contrast between the antero-dextral and postero-sinistral corners (Fig. 8E). The antero-dextral corner of the flange is never covered by blanket elements and in distal view shows delicate but distinct radiate sutures with anti-clockwise obliquity. On the postero-sinistral corner by contrast the V-unit elements are thin and are overlapped by blanket elements at very early growth stages, so they show smooth surfaces or a mass of fine rhombohedral crystallites and in any case clear continuity with blanket elements in the central area. Using these criteria it was possible to consistently orientate distal views at different growth stages.

From the oriented specimens it can be seen that growth commences at the outer edge of the proximal plate, where alternating V- and R-units indicate the proto-coccolith ring locus. The proximal plate develops rather simply, with progressive growth radially inward (Fig. 9). The flange is formed primarily by V-units, although it is overlapped by R-units of the blanket. It grows in a spiral, commencing at the flange origin near the anterior end of the coccolith. It then extends around the sinistral side of the proximal plate, forming an extension of the proximal plate, but with different crystallographic orientation. At the posterior end of the coccolith, however, the flange moves up the proximal plate to meet a tube structure which is located well inside the margin of the proximal plate. The flange continues to grow around the tube extending progressively up the dextral side and around the anterior end, where it expands to form the wing. Intermediate stage specimens show both the developing flange and the tube, both of which are formed of V-units. Where the tube is present it is connected to the margin of the baseplate by V-units, overlying the R-units of the baseplate. Thus the flange is formed from a single cycle of elements which develop very differently around the coccolith.

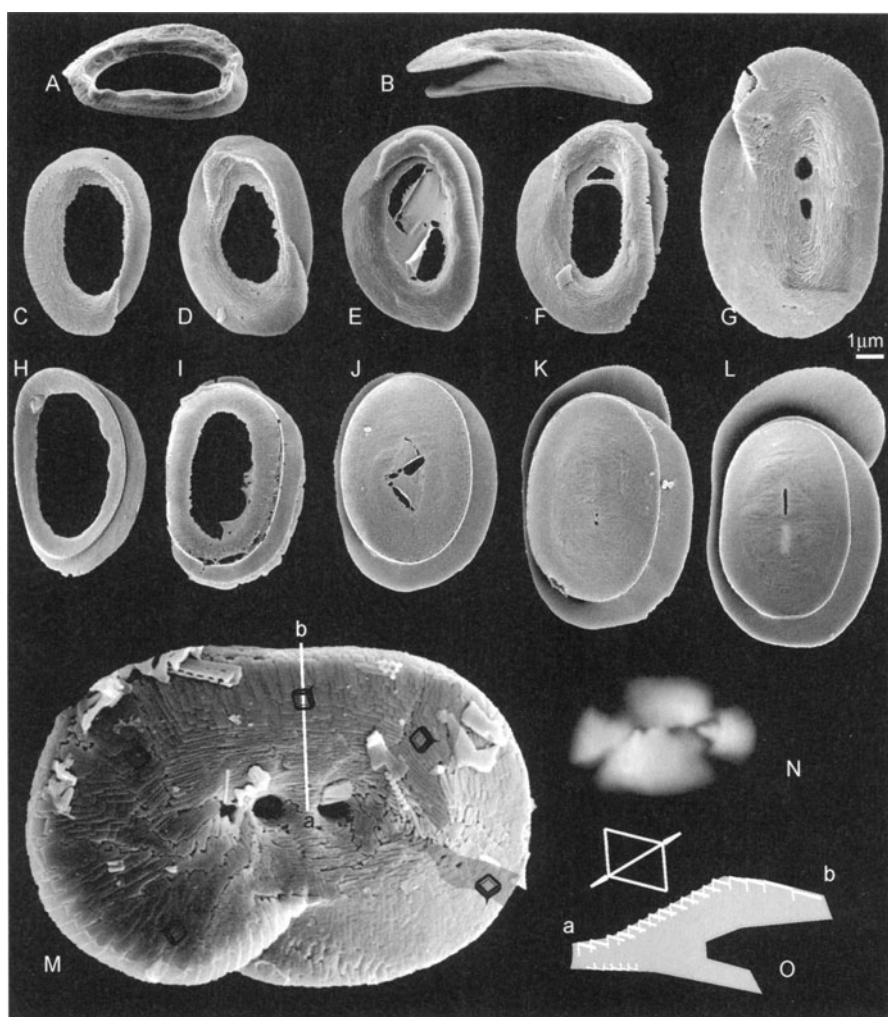


Fig. 9. *Helicosphaera carteri* growth and crystallographic orientation **A.** Early growth, dextral side view. **B.** Complete specimen sinistral side view. **C–G.** Distal view growth series, specimens orientated with anterior end toward top. **H–L.** Proximal view growth series. Note how flange growth proceeds clockwise. **M.** Slightly etched specimen with sutures between blanket elements opened. Symbols show calcite rhombs in projection parallel to one rhombic face. These have been used to identify crystal facets of blanket elements with c-axes oriented N-S and E-W, which would thus be in extinction in cross-polarized light, these areas have then been traced out and shaded. **N.** Light micrograph of specimen in the same orientation in cross-polarized light to show direct equivalence of inferences from crystal facets and light microscopy. **O.** Sketch cross-section along line a–b of Fig. 9M, rhombs in inferred orientation showing central area is formed of numerous small steps (resulting in needle-like facets) and flange of a few large steps (resulting in larger rhombic faces). More speculatively small steps shown on proximal plate, compare with Fig. 9B)

The blanket as noted above grows upward from the proximal plate and extends from the central area to cover most of the flange, so the V-units are often only exposed around the edge of the wing.

Syracosphaera pulchra

Syracosphaera pulchra differs from the other species considered here in having a polymorphic coccosphere with different exothecal, body, and circum-flagellar coccoliths (Fig 10C). Moreover, the coccoliths have relatively complex central areas formed of elements disjunct from the rim. It is therefore a useful representative of the more complex coccoliths which have attracted relatively little attention in terms of biomineralization. The morphology of *S. pulchra* coccoliths has been described by several workers (Halldal and Markali 1955; Black and Barnes 1961; Gaarder and Heimdal 1977) but most usefully by Inouye and Pienaar (1988), who showed that the coccolith was formed of three types of crystal-units. Based on light microscope observations Young et al. (1999) suggested that the rim was formed of V- and R-units while the central area laths had tangential c-axis orientation. Here we present evidence that this is the case, constituting a significant modification of the V/R model with obvious implications for inferring phylogeny.

Body coccoliths

As Inouye and Pienaar (1988) showed, the rims of *S. pulchra* body coccoliths are formed of two units: a lower one which forms most of the wall and the two lower flanges; and an upper one which forms the distal part of the wall and the distal flange (Figs. 10, 12). Inouye and Pienaar (1988) suggested from TEMs that the upper units extended between the lower units to the proximal surface, this is not obvious in SEMs of well-preserved specimens but on specimens where the upper unit has broken off a pore extending deep into the lower unit can be seen (Fig. 12 H–I). In the light microscope the two rim cycles can be observed in side views, and with a gypsum plate it is easily determined that the upper unit has sub-radial c-axes and the lower unit sub-vertical c-axes (Fig. 11F–G). The rim thus shows a normal V/R structure, with the proto-coccolith ring locus probably close to the base of the tube, although a clear alternation of crystal units is never seen in SEMs.

The radial laths are more problematic, our SEM observations confirm those of Inouye and Pienaar (1988) that the laths alternate around the inner margin of the rim with the lower rim elements but are separate from them (Fig. 10B). In the light microscope, these elements show distinct birefringence in plan view despite their thinness, so they must have sub-horizontal c-axes. With a gypsum plate the color distribution of the central part, formed from the laths, is opposite to that of the rim, indicating that they have a tangential distribution of the c-axes (Fig. 11D–E). This rather surprising result is supported by the lath morphology; on the proximal side the laths are sharp edged with two apparent crystal faces meeting at an accurate

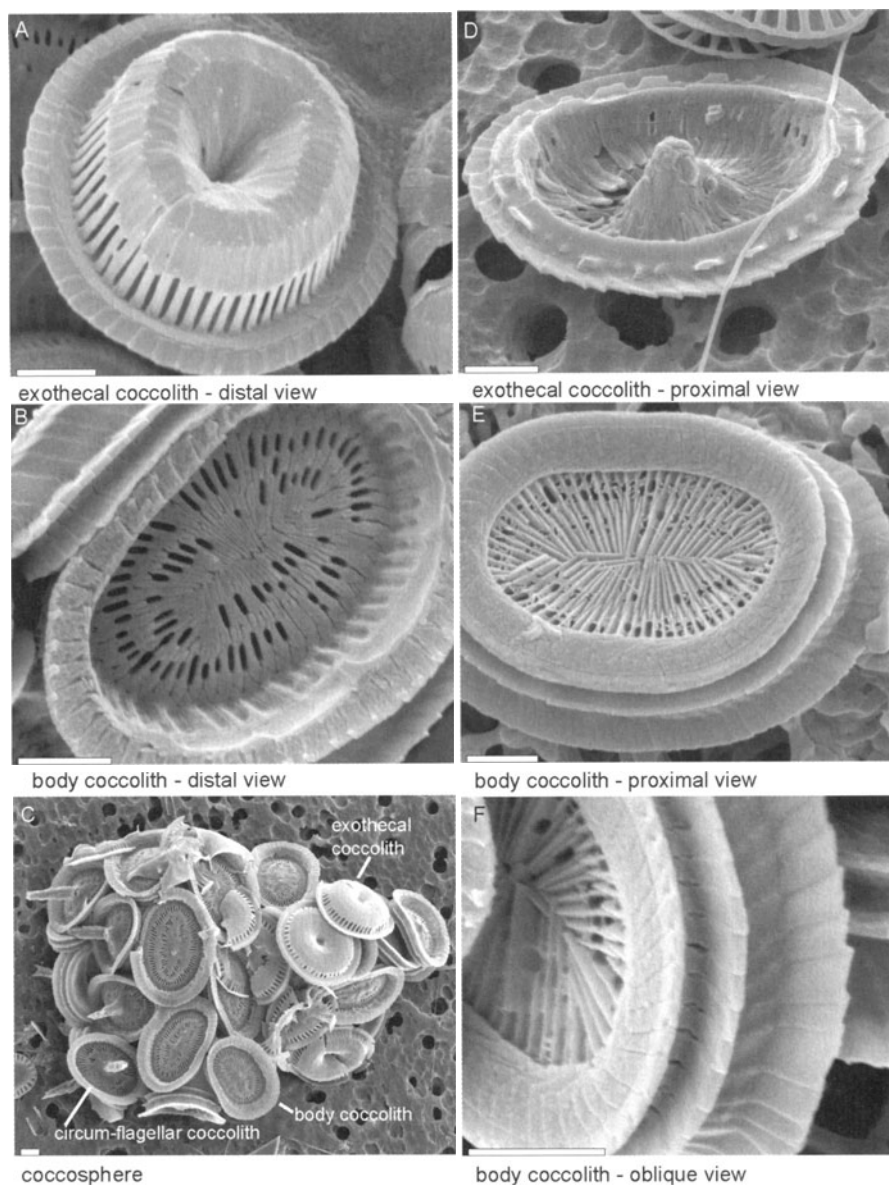


Fig. 10. *Syracosphaera pulchra* coccolith structure, plankton samples. Scale bars 1mm. **A–B.** Distal views of exothecal and body coccoliths. **C.** Coccosphere, showing disposition of the different coccolith types. **D–E.** Proximal views of exothecal and body coccoliths. **F.** Body coccolith in oblique proximal view. Note that laths are formed on this side of two crystallographic faces meeting at a sharp edge. Note also slits between proximal shield periphery and mid-shield sharp flanges, apparently showing extensions of the R-units to this level.

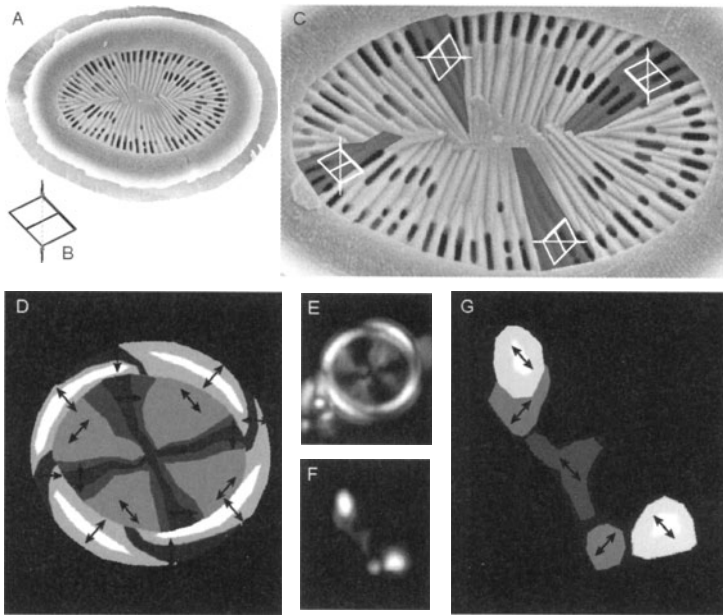


Fig. 11. *Syracosphaera pulchra* – endothecal coccoliths and their crystallographic orientation. **A–C.** SEM inference of crystallographic orientation of radial lath elements. **A.** proximal view of body coccolith. **B.** View of calcite rhombohedron in orientation such that an acute edge is parallel to the plane of view (from Fig. 1C), this must be the orientation of the central area laths if the crystallographic surfaces (cf. Fig. 10F) are rhomb faces, c-axis indicated by conical symbols. **C.** Enlarged view of central of specimen in A. Symbols rhombohedra as in B with sharp edge parallel to length of lath, as a result c-axis orientations are approximately tangential. Shading indicates laths with c-axes orientated N-S or E-W (and so in extinction in cross-polarized light), based on inferred orientation. **D–G.** LM inference of crystallographic orientations. **D–E.** Drawing and micrograph of a well-calcified body coccolith seen in proximal view (as determined by through-focusing) in cross-polarized light. In the drawing (D) c-axis orientations are indicated by double-headed arrow symbols, using also evidence from gypsum (1 λ) plate observations. The orientations are radial in the rim but tangential in the central area, and there is a very close correspondence of observed extinction pattern in the central area with that predicted from SEM observations (C). **F–G.** Drawing and micrograph of a well-calcified circum-flagellar coccolith seen in side view in cross-polarized light. In the drawing (G) c-axis orientations are indicated by double-headed arrow symbols, using also evidence from gypsum (1 λ) plate observations. Note that orientations are radial in the rim but tangential in the central area.

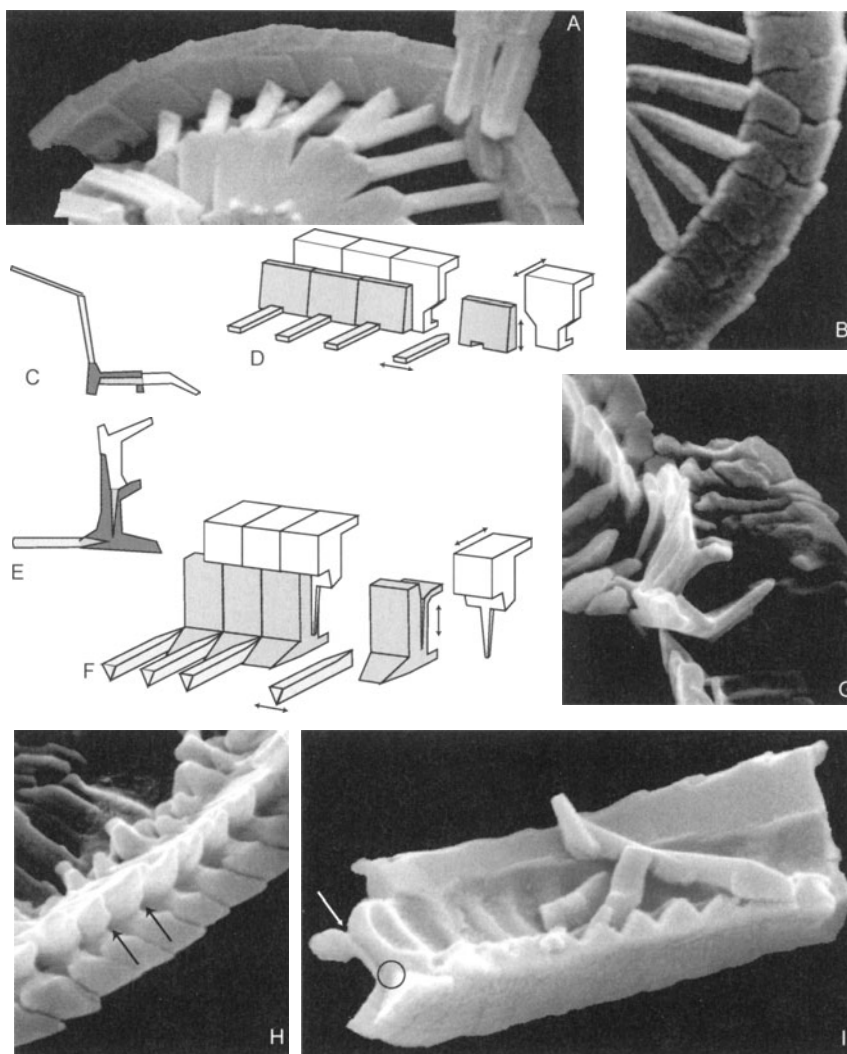


Fig. 12. *Syracosphaera pulchra* rim structure. **A–D.** Exothecal coccolith structure. **A.** Oblique-distal view showing lath elements and two element types in wall. **B.** Proximal view of collapsed specimen showing alternating element types in wall. **C.** Summary cross-section in natural orientation. **D.** Simplified 3-D model shown in orientation to facilitate comparison with body coccoliths (F); double-headed arrows indicate crystallographic orientations, based on analogy with body coccoliths. **E–I.** Body coccolith structure. **E.** Summary cross-section. **F.** 3-D model, double-headed arrow symbols crystallographic orientations, from Fig. 11. **G.** Oblique view of partially disintegrated wall showing V-unit form; R-units detached to left. **H.** Distal view of V-units from which R-units have been detached; arrows indicate holes between V-units suggesting that R-units extended between them. **I.** Oblique-proximal view of wall; arrow indicates tube extending into structure; circle: inferred locus of proto-coccolith ring.

angle (Fig. 10E–F, Black and Barnes 1961). If these are rhombohedral faces, then the c-axis orientation must be nearly tangential (Fig. 11A–C). The consistency of the light microscope and SEM observations (Fig. 11C–D) thus indicates rather strongly that in *S. pulchra* there are not two crystal unit types but three, with sub-vertical, sub-radial and sub-tangential orientations, relative to the local rim orientation. The number of units in each cycle is the same and they all originate from near the base of the rim (Fig. 12I). Therefore in *S. pulchra* we can envisage a proto-coccolith ring similar to the typical V-/R- proto-coccolith ring but with three types of nuclei. This structure is summarized in Fig. 12E–F.

Exothecal coccoliths

The exothecal coccoliths of *S. pulchra* have a very different shape to the body coccoliths (Fig. 10A, D), but as shown by Inouye and Pienaar (1988) their structure is similar (Figs. 10A–D, 12C–F). Essentially, in the exothecal coccoliths, the rim has been rotated through 90° and the central area extruded into a domal shape. In well-formed exothecal coccoliths the similarity of rim form to the body coccoliths is particularly striking, including development of three flanges (Fig. 10C–D).

The domal shape of the exothecal coccoliths highlights the fact that there are multiple lath cycles with each cycle formed of separate crystal units. In the body coccoliths multiple cycles are apparent (Fig. 10B, E), but since they are co-planar it is not certain that they are separate crystal units, even if this seems likely. In the exothecal coccoliths, while the successive lath cycles curve over to form the domal shape (Figs. 10A, D, 12C) hence they must be separate crystal units. The neat interrelationship of the outermost laths with the rim units indicates that these laths must have nucleated at the rim, however, the other lath cycles must have nucleated separately. The simplest prediction is that the lath cycles grow inward from the rim with crystallographic orientation of the inner cycles being influenced by the outer cycles, as tentatively suggested for holococcoliths (Young et al. 1999). However, we have not been able to observe clear growth sequences to substantiate this prediction.

Discussion

Growth regulation

Two consistently different types of element surfaces can be seen, even within heterococcolith rims showing normal V/R structure, and these probably reflect different crystal-growth regulation processes:

a. Non-crystallographic surfaces. These surfaces are formed when growth is confined to narrow crystal fronts so that the coccolith surface is formed accretively, and no growth occurs on the surface after deposition. This pattern is seen in *Gephyrocapsa* and *Emiliana* and on the proximal surfaces of *Coccolithus*, *Calcidiscus*, *Umbilicosphaera*, and *Helicosphaera*. With this growth style individual

crystal units often develop complex three-dimensional shapes but contact planes between elements form more or less perpendicular to the coccolith surface and as a result elements are well distinguished. With this type of growth the crystallography almost certainly strongly influences the direction of growth but crystal faces are only developed at the leading edge, and often are lost on further growth. The absence of growth on the other surfaces strongly implies blocking of surfaces by organics, and the morphologies developed are unstable in inorganic systems.

b. Rhombohedral crystal faces. These faces are inorganically stable and formed by growth in layers over large surfaces. In *C. pelagicus*, these surfaces are atomically flat (Henriksen et al. 2003, in press). This type of growth is not confined to a narrow growth front but occurs across the entire exposed surface of the crystal unit. This pattern is seen on the distal surfaces of *Coccolithus*, *Umbilicosphaera*, *Calcidiscus*, and *Helicosphaera*. It is not shown by *Gephyrocapsa* or *Emiliania*. With this growth type the gross morphology of the crystal units is typically simple but small scale growth occurs in crystallographically determined directions resulting in superficial complexities ranging from the bicyclic appearance of *U. foliosa*'s distal shield (Fig. 4B) to the multifaceted needle-like surface of *Helicosphaera* blanket elements (Fig. 9M–N).

In both growth styles there is plainly interaction in biomineralisation between the crystallographic growth directions (generally either parallel to the c-axis or perpendicular to a rhombohedral face) and the developing vesicle in controlling shape and structure. The development of *Helicosphaera* provides further perspectives on how these processes must interact. The proto-coccolith ring appears to be essentially symmetrical, although probably with chirality of nucleation, as in *E. huxleyi* (Didymus et al. 1994). Growth from this ring is, however, asymmetric with initially growth of V-units occurring outward on one side and inward and upward on the other. Subsequently it seems that a growth front develops at the leading edge of the flange and sweeps clockwise round the coccolith forming the flange. This asymmetric growth can only be produced by active cellular regulation of the growth location and direction. Nonetheless there is no additional nucleation involved. Furthermore, crystal growth directions are maintained around the flange – with consistent clockwise obliquity seen in proximal view (Fig. 8A). The consistency of element elongation direction around the flange with its highly variable shape can only be a product of a preferential crystal growth direction.

Systematics and coccolith structure

The taxa sampled here include three relatively closely related genera, *Coccolithus*, *Calcidiscus* and *Umbilicosphaera* and three others which are representative of very disparate groups, *Helicosphaera*, *Syracosphaera* and *Gephyrocapsa*. As is evident from the discussion and Fig. 1, the only significant homology between all the disparate groups is growth of the rim from a proto-coccolith ring with V-/R-alternation. Almost all other aspects of coccolith structure are so different that they barely require further comment. It is, however, worth noting that these differences are much stronger than might be guessed from a casual inspection of elec-

tron micrographs which only show morphology not crystallographic orientations, but do result in very strong differences in appearance in cross-polarized light. As a result the high level taxonomy of coccolithophores founded on LM observations is more robust than is often realized. For instance, it is not uncommon to see the Noelaerhabdaceae coccoliths (shown by *Gephyrocapsa* and *Emiliana*) being characterized as placoliths with a grill, as opposed to Coccolithaceae coccoliths being characterized as placoliths with an open central area. This is not incorrect, but omits mention of the basic crystallographic differences between the coccoliths, which indeed make it likely that the placolith morphology is homoeomorphic rather than homologous between the two groups.

The genera *Coccolithus*, *Calcidiscus* and *Umbilicosphaera* provide a useful case-study in the relationship of coccolith structure to phylogeny at a closer level; they belong to two families in the current classification and probably diverged within the Cenozoic. Within this group of coccoliths the basic crystallography is similar with V-units forming the distal shield and R-units the proximal shield, but there are also large differences. There is a remarkable contrast between Fig. 4, which shows the exposed surfaces of these coccoliths, and Fig. 6 which shows detached shields exposing normally concealed surfaces. On the exposed surfaces (Fig. 4) there are numerous differences between these three genera, indeed it is difficult to find characters shown by all three genera. By contrast on the concealed surfaces (Fig. 6) the similarities are predominant and the structural differences appear trivial. This contrast can be explained by the observation that the proto-coccolith ring locus is situated within the tube in these genera. Growth directly from the proto-coccolith ring is conservative between the genera and produces the surfaces seen on the detached shields. Further from the proto-coccolith ring differences accumulate, producing the variation seen between the genera on the exposed surfaces.

A more subtle issue highlighted by this set of examples is that similar structures can evidently appear homoeomorphically in closely related taxa. The clearest example is the two-layered structure in the proximal shields of *C. pelagicus* and *U. foliosa* (Fig. 4D–E). Another example is provided by the bicyclic distal shield seen in *U. foliosa* (Figs. 4B, 5) and occasionally in *Calcidiscus* (e.g. malformed specimen seen in upper left of Fig. 4C). In both cases, the likely explanation of independent occurrence of similar structures is that they result from interaction of inorganic crystal growth (the directions of which are determined by the nucleation) with surface topography determined by the coccolith vesicle. If similar topographies recur in closely related taxa then similar element morphologies will result. So these structures reflect underlying homology in crystal orientation, but may or may not be expressed in particular species, depending on rather trivial differences in surface topography. This complicates definition of characters for phylogenetic analysis and is one reason why cladistic analysis of coccolith morphology has not been successfully attempted to date. Conversely, the fact that small differences in surface topography can produce large changes in suture pattern is certainly convenient for species-level taxonomy.

Nucleation modes

The V/R model of heterococcolith nucleation has proven very successful for interpreting rim structures (e.g. Young et al. 1992; Kleijne 1993; Young et al. 1999; Marsh 1999) and indeed fits well the species described here. Nonetheless it has been noted that many central area structures do not easily fit this model (e.g. Young 1994; Young et al. 1999). This is especially true of the Syracosphaeraceae, Rhabdosphaeraceae and Calciosoleniaceae all of which typically show radial lath cycles that appear to be disjunct from the rim, i.e. separately nucleated, and many of which show further cycles of disjunct elements within the central area (e.g. Kleijne 1992; Cros and Fortuño 2002). *Syracosphaera pulchra* is the only member of this species-rich group which produces coccoliths large enough for light microscopic determination of crystallographic orientations of the radial lath elements, but otherwise appears to be representative of the group. So the results determined here are significant. The basic conclusions are: (1) that the rim has a normal V/R-type structure; (2) that the radial laths are an additional, disjunct cycle of elements with sub-tangential c-axis orientations, "T-units"; (3) that the radial laths alternate with the basal (V-unit) elements around the inner margin of the rim; (4) that around the rim the ends of the T-unit laths must be very close to the locus of the proto-coccolith ring. This suggests that the nucleation pattern in *S. pulchra* consists of sets of three nuclei with respectively sub-vertical, sub-radial and sub-horizontal orientations, a significantly more complex biomineralisation structure than the typical heterococcoliths.

The very numerous other species of the Syracosphaeraceae have similar basic structures; in SEM lath cycles are universally seen in the central area and in most cases high resolution SEMs show that two cycles in the rim are present and that the lath elements alternate with them. In proximal views laths often show similarly sharp edges to those in *S. pulchra* suggesting tangential c-axis orientations. Therefore we predict that the *S. pulchra* type structure is common for the Syracosphaeraceae. Moreover the structure of the Rhabdosphaeraceae and Calciosoleniaceae are obviously similar, in particular they definitely have rims with a normal V/R-type structure and in addition a radial lath cycle. So it seems reasonable to predict that these three families are closely related. Molecular genetics (Saez et al. this vol.) support the grouping of the Syracosphaeraceae and Rhabdosphaeraceae.

Conclusion

Coccolith biomineralisation is a complex process and the interaction of inorganic crystal growth and organic regulation is still only partially understood. However, a key input to developing such understanding has been analysis of coccolith structure and growth and comparative study of related coccoliths. In this work an understanding of phylogeny and diversity has been a powerful tool in studying biomineralisation. Conversely a developing knowledge of the processes which

form coccoliths is allowing us to better interpret coccolith morphology and maximize phylogenetic data retrieval from them. As in many other areas of coccolithophore research, integration of data from different sources, notably in this case molecular genetics and biomineralisation studies, has multiplied the value of results from each study.

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The Laboratory Culture of Coccolithophores

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Summary

Coccolithophores have now been cultured for over 75 years. This period has witnessed progressive improvements in culturing techniques and a steady increase in the number of coccolithophores which have been brought into culture. In certain respects our knowledge of the biology of coccolithophores is as advanced as that in any other group of microalgae and this is due in large part to culture experiments which have focused on two easily cultured species, *E. huxleyi* and *P. carterae*. A continued emphasis on *E. huxleyi* in culture investigations linked to global change issues is to be expected. There is, however, a definite lack of comparative culture data for other coccolithophore species and hence our comprehension of the biodiversity within this group is currently rather limited. There is considerable potential for addressing this situation by further exploiting existing cultures of other coccolithophore species currently maintained in collections around the world. While coccolithophores do not appear to be intrinsically more difficult to culture than other microalgal groups, the fact remains that the majority of coccolithophores have not yet been successfully cultured. There is a strong habitat and phylogenetic bias in our present culture collections: to date, culture techniques have been quite successful in culturing the more *r*-selected species from eutrophic and intermediate habitats, but there are few cultures of *K*-selected species from oceanic oligotrophic habitats. This may be because no one has ever tried to culture these species, but is probably also related to limitations inherent with current culturing techniques and culture media. *K* medium is perhaps the best available option for successful culturing of oceanic coccolithophores, but further advances, including the reduction in concentration of macro- and micro-nutrients, and the possible use of organic nutrients, should also be considered.

Introduction

Unicellular planktic algae form the basis of the marine food chain and due to the huge size of the world ocean and hence their sheer number, they are responsible

for an important part of global primary production and influence biogeochemical cycles on a global scale. The key to explaining present and past phytoplankton biodiversity and distributions, and thus also predicting future responses to global change, is information on the basic biology of individual species (e.g. morphological, ultrastructural, genetic, biochemical, physiological, behavioral and life history characteristics) and understanding of how these biological features interact with environmental conditions to promote the selection of one species over another. The extraordinary diversity and minute size of phytoplankton, together with the relative inaccessibility of their habitats, mean that the amount of information on individual species that can be obtained by *in situ* studies is restricted. Developing techniques for the automated sorting of phytoplankton cells or for molecular analysis of bulk samples promise an increasing emphasis on species-specific studies in the natural environment, but these techniques have certain inherent limitations, not the least of which is the requirement for *ex situ* calibration. The principal source of much of the species-specific basic biological information have been up until the present time, and are likely to remain for the foreseeable future, observations and experimentation on microalgae maintained *ex situ* in laboratory cultures. In a broad sense, culturing refers simply to *ex situ* growth, but the modern concept of microalgal culturing can be defined as the perpetual *ex situ* maintenance of monoalgal populations. In this context, the use of clonal cultures (i.e. cultures originating from the isolation of a single cell) has the particular advantage of permitting the non-simultaneous investigation of multiple aspects of the biology of the species, with the theoretical assurance that all studies are conducted on the same biological entity.

In terms of abundance, the coccolithophores are considered to be one of the three main marine phytoplankton groups (along with the diatoms and the dinoflagellates). This group of calcifying haptophytes includes two phylogenetically distant species, *Emiliania huxleyi* and *Pleurochrysis carterae*, which are among the most extensively studied of all microalgal species in culture experiments. Paradoxically perhaps, it is often stated that coccolithophores are difficult to culture. It is a fact that of the approximately 200 extant species of coccolithophore only a small fraction have ever been successfully cultured, and this has introduced a strong bias in our actual knowledge of the biology of this group. The first aim of this chapter is to review the history of the laboratory culture of coccolithophores and of the utilization of this resource, and in this context cultured strains of coccolithophore species currently being maintained in the main service collections around the world are listed. Secondly, perspectives for the future exploitation of the existing culture resource are assessed, and subsequently, on a more practical level, an overview of the methods involved in culturing microalgae is presented with special reference to examples from the literature and from our own experiences of how these methods have been applied to the isolation and culture of coccolithophores. Finally, prospects for increasing the taxonomic range of coccolithophores maintained in culture are discussed.

A brief history of the culture of coccolithophores

The early history of microalgal culturing was reviewed by Allen and Nelson (1910). The first reported culture of marine phytoplankton was in 1890 by P. Miquel. Many of the concepts and methods that are routinely used in microalgal culturing today are in fact remarkably similar to those developed in the late nineteenth and early twentieth centuries. These included the adoption from microbiological research of sterile techniques, the utilization of single cell isolation methods using a capillary pipette or serial dilutions, and the establishment of axenic algal cultures. Simple culture media recipes (both liquid and agar) were developed, based on the addition of varying concentrations of macronutrients and iron to coastal seawater. Early workers realized that distilled water, seawater, glassware and chemicals were all sources of impurities, implying that unknown contaminating trace nutrients were being added inadvertently to the culture medium, many of which were suggested to contribute to better algal growth (although the concept of trace metal toxicity was also described), and the importance of pH regulation and vitamins had also already been recognized (Allen and Nelson 1910). Early efforts were successful in the culture of inshore coastal species, mainly diatoms and chrysophytes, all of which have high environmental tolerances. This was in part due to limitations of the culture media, but also due to focus on the easily accessible coastal environment for sampling. An early innovation saw the addition of soil extract, beneficial to the growth of many coastal species, to culture medium recipes (Schreiber 1927).

Coccolithophores were first correctly identified as unicellular algae in the 1870s and the early twentieth century saw intensive systematic work on extant (most often fixed, but sometimes living) and to a lesser extent fossil material (see review by Siesser 1994). Aspects of the cytology of coccolithophores were described for living coccolithophores and Klebs (1893) even reported division of cells of the freshwater coccolithophore *Hymenomonas roseola*. This could arguably be considered to have been the first coccolithophore culture (i.e. *ex situ* growth), but these unenriched mixed samples were not maintained past this division stage. Several decades passed before coccolithophores were first maintained for extended periods of time in monoalgal laboratory cultures. Non-clonal strains of both *Ochrosphaera neapolitana* (Schussnig 1930; Schwarz 1932) and *Hymenomonas roseola* (Schiller 1930) were obtained by serial sub-culture of enriched mixed samples. Schiller (1926) also studied *Pontosphaera* (= *Emiliania*) *huxleyi* (although from the illustrations this designation is doubtful), *Syracosphaera pulchra*, *Syracosphaera* (= *Coronosphaera*) *mediterranea* and the holococcolithophore *Calyptosphaera oblonga* from enriched mixed samples, but long-term cultures of these species were not established. (N.B. current taxonomic nomenclature is substituted for outdated terminology hereafter). Schiller (1930) and Kamptner (1941) both stressed the importance of being able to study coccolithophores in culture, and the latter author remarked that experiments to culture coccolithophores had not been very successful up until that time and consequently little was known about conditions and rules of the different reproduction proc-

esses. The first monoalgal cultures of *E. huxleyi*, which were attained by the serial dilution method, were reported by Braarud (1945), while the first clonal coccolithophore cultures were reported shortly afterwards by Braarud and Fagerland (1946) who used capillary tubes to isolate *Pleurochrysis carterae* cells into culture.

In the period between 1925 and 1950, light microscope investigations of live samples and cultured coccolithophores contributed to the taxonomic cataloguing and notably to the cytological characterization of species and resulted in the first investigations into the physiology and life cycles of this group. In the early 1950s the development of powerful new microscope techniques, including cross-polarized and phase contrast illumination for the light microscope and transmission electron microscopy (TEM), vastly improved the capability to observe microalgae. During the 1950s and 1960s the Norwegian group led by T. Braarud as well as M. Parke and colleagues in England were at the forefront of investigations into numerous aspects of the morphology and fine structure of haptophytes, but whereas several non-calcifying haptophytes were brought into culture for the first time during this period, most investigations on cultured coccolithophores were conducted on species for which cultures already existed. The initial focus was on taxonomic revision based on detailed observation of the structure of the coccoliths of several species, including most of those previously cultured (e.g. *E. huxleyi* and *P. carterae*, Braarud et al. 1952; *H. roseola*, Braarud 1954). At around the same time, details of the life history of two newly cultured species, *Pleurochrysis scherffellii* (Pringsheim 1955) and *Coccolithus braarudii* (both hetero- and holococcolith bearing life cycle phases) (Parke and Adams 1960) were reported (N.B. *C. braarudii* is the large temperate form of *C. pelagicus*, recently elevated to species level by Sáez et al. 2003). The latter study was the first report of a holococcolith-bearing stage being maintained in culture, albeit that the strain had initially been isolated in the heterococcolith-bearing stage, a subsequent phase change leading to the presence of both stages in cultures. Parke and Adams (1960) stressed the importance of the study of cultures for gaining valuable information on the behavior and life history of these organisms. As an example, the characterization in cultures of the peculiar third appendage observed in motile coccolithophores as a haptonema (Parke et al. 1955; von Stosch 1958; Parke and Adams 1960) led to the removal of the coccolithophores from the Chrysophyceae and their placement in a new class, termed the Haptophyceae (Christensen 1962), where they joined other haptonema-bearing (but non-calcifying) genera, notably *Prymnesium* and *Chrysochromulina*. The English and Norwegian groups were among the first to conduct detailed TEM ultrastructural investigations of coccolithophores, initially using existing cultures (*C. braarudii* holococcolith-bearing phase, Manton and Leedale 1963; *C. braarudii* heterococcolith-bearing phase and *P. carterae*, Manton and Leedale 1969; *H. roseola* and *P. carterae*, Manton and Peterfi 1969; *E. huxleyi*, Klaveness 1972).

Up until the 1960s the majority of the research effort had been directed at taxonomic description and cytological characterization of marine algae, but around this time an increasing emphasis was placed on attempting to understand species distributions and seasonal population successions. Interest in different aspects of

the physiology of cultured marine algae stimulated improved analytical techniques (nutrient assays for example) and more rigorous culture methods, most notably the development of new culture media in which soil extract was replaced by specific elements (e.g. Provasoli et al. 1957; Guillard 1975), and also standardization of the use of clonal cultures. The large body of experimental work on the physiological ecology of coccolithophores, initiated in the 1960s notably by E. Paasche and colleagues in Norway, has been summarized in the excellent reviews of Brand (1994) and Paasche (2002) and here only the main themes and the introduction of newly cultured species will be highlighted. Early autecological experiments on nutrient, light and temperature preferences generally adopted a comparative approach using a selection of microalgal species from different classes, with the heterococcolith-bearing stages of *E. huxleyi* and *P. carterae* most often representing the coccolithophores. Through the 1970s and 1980s much interest was shown in understanding the process of coccolith formation. In this context, experimental work also centered around *E. huxleyi* (Klaveness 1972; Klaveness and Paasche 1979; Borman et al. 1982; Westbroek et al. 1984, 1986, 1989) and *Pleurochrysis* (Outka and Williams 1971; Pienaar 1971, 1976; Van der Wal et al. 1983a, 1983b, 1987, Westbroek et al. 1986). Rowson et al. (1986) also investigated coccolith production in cultures of the holococcolith-bearing phase of *C. braarudii* and (unknowingly) commented on cultures of *C. pelagicus* from the Plymouth culture collection which had been isolated some years earlier. In the early 1980s, L. Brand and colleagues isolated several previously uncultured coccolithophore species, namely *Gephyrocapsa oceanica*, *Calcidiscus leptoporus*, *Umbilicosphaera hultburtiana* and *U. sibogae*, and introduced them into their eco-physiological research (Brand and Guillard 1981; Brand 1981, 1982; Brand et al. 1983; Brand 1984). Brand (1994) also mentions unpublished results on cultures of *Syracosphaera pulchra*, *Reticulofenestra sessilis* and *Scyphosphaera apsteinii*.

The 1970s and 1980s saw a steady increase in the body of ultrastructural information, notably through the work of I. Inouye, R. Pienaar and co-workers in Japan and South Africa, and P. Gayral and J. Fresnel in France. Certain of these investigations were conducted on previously cultured species: *O. neapolitana* (Gayral and Fresnel-Morange 1971), *P. carterae* (Beech and Wetherbee 1988), *Pleurochrysis* sp. (Inouye and Pienaar 1985). This line of research also proved, however, the stimulus for the isolation of several previously uncultured (and in some cases previously undescribed) species: *Calyptrosphaera sphaeroidea* (the first holococcolithophore to be isolated in this phase and maintained in pure culture; Klaveness 1973), *Hymenomonas coronata* (Mills 1975), *H. lacuna* (Pienaar 1976), *Hymenomonas globosa* and *Pleurochrysis roscoffensis* (Gayral and Fresnel 1976), *Jomonolithus littoralis* (Inouye and Chihara 1983), *Pleurochrysis pseudoroscoffensis* (Gayral and Fresnel 1983), *Umbilicosphaera foliosa* (Inouye and Pienaar 1984), *Cruciplacolithus neohelis* (Fresnel 1986, Kawachi and Inouye 1994), *Syracosphaera pulchra* (Inouye and Pienaar 1988), and *Pleurochrysis placolithoides* (Fresnel and Billard 1991). During these two decades the microanatomy of many cultured non-calcifying haptophytes was also being characterized, often by the same workers, and much key information for the understanding of the biology and ecology of the haptophytes as a whole was forthcoming. Phylogenetic trends

in the structure of features such as the flagellar root system, Golgi-related scale formation and pyrenoid structure were (in most cases tentatively) suggested (see reviews by Green and Hori 1994; Leadbeater 1994; Pienaar 1994). Since the early 1990s, however, studies involving ultrastructural characterization of coccolithophores (and indeed microalgae as a whole) have been less common, with only one species that had not previously been examined, the newly discovered holococcolithophore *Calyptrosphaera radiata*, being described from culture material by Sym and Kawachi (2000). It is perhaps no coincidence that this period has seen the rise in prominence of genetic methods for determination of relationships between taxa. Since it has not yet proved possible to extract DNA from single coccolithophore cells, cultures have been a prerequisite for molecular genetic studies. The molecular phylogeny of the Haptophyta published by Edvardsen et al. (2000) included several previously cultured coccolithophores, members of the families Noelaerhabdaceae, Pleurochrysidaceae and Coccolithaceae, while that of Fujiwara et al. (2001) included notably one species which had not previously been reported in culture, *Helicosphaera carteri*.

In the early 1990s, the European-based EHUX project (1992–1995) was among the forerunners of an exponential increase in the number of multidisciplinary studies focusing on the ubiquitous bloom forming species *E. huxleyi*. Much of this research was culture based, and culture experiments ever more complex in design and scope have resulted in key advances in our understanding of the biology of this species, including aspects such as the relationship between photosynthesis and calcification, nutrient and trace metal physiology, pigment composition, DMS production, alkenone carbon-bond saturation and carbon isotope composition (see for example reviews by Simó 2001; Laws et al. 2001; Paasche 2002). This period has seen the first concerted application of chemostat (continuous culture) techniques and also the first mesocosm experiments involving a coccolithophore. The latter typically involve measurements of *in situ*, natural, mixed assemblages in semi-controlled conditions, and as such are not considered further here.

During the CODENET (Coccolithophore Evolutionary Biodiversity and Ecology Network) project (1998–2001) a targeted collection and isolation programme led to the initiation of multiple strains of six keystone genera (*Gephyrocapsa*, *Coccolithus*, *Calcidiscus*, *Syracosphaera*, *Umbilicosphaera* and *Helicosphaera*) which had been selected for integrated biological (mainly culture-based), physiological (e.g. Quinn et al. 2003) and paleontological research. Isolation is an intrinsically serendipitous activity, and cultures of several other species were also isolated as and when seen in samples, some of which had previously been cultured on at least one occasion (*E. huxleyi*, *Jomonlithus littoralis*, *Scyphosphaera apsteinii*) and others which were successfully cultured for the first time (*Oolithotus fragilis*, *Coronosphaera mediterranea*, *Algirosphaera robusta*, *Helicosphaera hyalina*). As a result of phase changes in some culture strains, pure cultures of the haploid (non-calcifying or holococcolith-bearing) phase of several species were also initiated, many for the first time (e.g. Geisen et al. 2002).

This brief review provides some idea of the major contribution that observation and experimentation on cultured coccolithophores have made towards attaining

Table 1. List of all previously cultured coccolithophore species (taxonomy from Jordan and Green 1994, with some modifications according to recent findings, notably those of Sáez et al. 2003). N.B. Asterisks denote those species for which both life cycle phases have been cultured.

Family	Species cultured
Noelaerhabdaceae	<i>Emiliania huxleyi</i> * (types A, B, C and R) <i>Gephyrocapsa oceanica</i> * <i>Reticulofenestra sessilis</i>
Pleurochrysidaceae	<i>Pleurochrysis carterae</i> *, <i>P. dentata</i> *, <i>P. elongata</i> , <i>P. gayraliae</i> *, <i>P. placolithoides</i> *, <i>P. roscoffensis</i> *, <i>P. scherffellii</i> *
Hymenomonadaceae	<i>Ochrosphaera neapolitana</i> * <i>Hymenomonas globosa</i> , <i>H. lacuna</i> *, <i>H. coronata</i> *, <i>H. roseola</i> <i>Jomonolithus littoralis</i>
Coccolithaceae	<i>Calcidiscus leptoporus</i> *, <i>C. quadriperforatus</i> <i>Umbilicosphaera foliosa</i> , <i>U. sibogae</i> , <i>U. hultburtiana</i> <i>Oolithotus fragilis</i> <i>Coccolithus pelagicus</i> , <i>C. braarudii</i> *
Syracosphaeraceae	<i>Syracosphaera pulchra</i> * <i>Coronosphaera mediterranea</i> *
Rhabdosphaeraceae	<i>Algirosphaera robusta</i>
Pontosphaeraceae	<i>Scyphosphaera apsteinii</i>
Helicosphaeraceae	<i>Helicosphaera carteri</i> , <i>H. hyalina</i>
Calyptrosphaeraceae	<i>Calyptrosphaera sphaeroidea</i> , <i>C. radiata</i> , <i>Helladosphaera</i> sp.

our current state of knowledge of the taxonomy and biology of this group. By means of a summary of the history of the laboratory culture of coccolithophores, all species which have been successfully cultured on at least one occasion since the first reported culture of *Ochrosphaera neapolitana* by Schussnig (1930) are listed in Table 1.

Existing cultures of coccolithophores

Table 2 summarizes all species which are currently being maintained in those culture collections world-wide for which recently updated information is available either in the form of a WWW site or a published catalogue (information correct as of June 2003). The CODENET collection has been integrated into the ALGO-BANK collection at the University of Caen, France. Strains in the collections listed are available generally for a small fee which contributes to the operational costs of these systematically under-funded collections, and hence to the continued maintenance of this important resource. A current theme of discussion among the main service collections is collation of strain information in integrated data bases, and such information may become available in the near future.

Table 2. Coccolithophore cultures currently maintained in the main service culture collections world-wide.

Species	No. strains	A	B	C	D	E	F	G	H	I	J	K	L
<i>A. robusta</i>	1						1						
<i>C. leptoporus</i>	22						20			2			
<i>C. quadriperforatus</i>	14						14						
<i>Calyptrosphaera</i> sp.	3						1		1				
<i>C. sphaeroidea</i>	6				1		1			4			
<i>C. radiata</i>	3						1			1		1	
<i>C. braarudii</i>	7					1	6						
<i>C. mediterranea</i>	1						1						
<i>C. neohelis</i>	9	1		1	1		2		2	1			1
<i>E. huxleyi</i>	305	17	9	6	1	209	33	4	3	5	3		14
<i>G. oceanica</i>	86	2				3	74	1	2	3			2
<i>Gephyrocapsa</i> sp.	2								2				
<i>H. carteri</i>	9						9						
<i>H. hyalina</i>	1						1						
<i>Helladosphaera</i> sp.	2						1		1				
<i>H. coronata</i>	3						2			1			
<i>H. globosa</i>	1					1	1						
<i>J. littoralis</i>	1						1						
<i>O. neapolitana</i>	21	6	3		1	3	3				1		5
<i>Ochrosphaera</i> sp.	3					3			3	2			
<i>O. verrucosa</i>	7	1	1		1		1						1
<i>O. fragilis</i>	21						18				3		
<i>P. carterae</i>	22	5	1	1	2	6	1				1	1	4
<i>P. dentata</i>	1						1						
<i>P. elongata</i>	6		1	1		1	2						1
<i>P. gayraliae</i>	6		1		1		2				1		1
<i>P. placolithoides</i>	4	1		1			1						1
<i>P. roscoffensis</i>	7	1					4						1
<i>P. scherffellii</i>	3	1	1				1						
<i>Pleurochrysis</i> sp.	18	3	1	2		8			2				2
<i>S. apsteinii</i>	3						3						
<i>S. pulchra</i>	13						13						
<i>U. hultburtiana</i>	1						1						
<i>U. foliosa</i>	2						1			1			
<i>U. sibogae</i>	6						3			3			
Total	620	38	18	12	8	235	224	5	16	23	6	2	33

A: CCMP (USA) <http://ccmp.bigelow.org>; B: CCAP (UK) <http://www.ife.ac.uk/ccap/>; C: CCCM (Canada) <http://www.botany.ubc.ca/cccm>; D: UTEX (USA) <http://www.bio.utexas.edu/research/utex/>; E: Plymouth Culture Collection (UK) <http://www.mba.ac.uk/culture-collection/culture-collection.htm>; F: ALGOBANK Caen (France) <http://www.unicaen.fr/unicaen/ufr/ibba/lbbm/algobank>; G: RCC Roscoff (France) <http://www.sb-roscoff.fr/Phyto/collect.html>; H: MBIC (Japan) <http://seasquirt.mbio.co.jp/mbic/index.php?page=top>; I: NIES (Japan) <http://www.nies.go.jp/biology/mcc/home.htm>; J: CSIRO (Australia) <http://www.marine.csiro.au/microalgae>; K: EPSAG (Germany) <http://wwwuser.gwdg.de/~epsag/Web/>; L : Others

Workers conducting culture experiments using strains procured from culture collections should always take personal responsibility for (a) verifying the taxonomic identity and purity of the culture (or having this done by an expert), and (b) ensuring that current taxonomic nomenclature is applied. The mis-identification of culture strains is at best confusing and in reality usually invalidates the interpretation of results. To our knowledge, the vast majority of the coccolithophore strains listed in Table 2 were given the correct taxonomic designation at the time of identification. It is very difficult, however, for culture collection curators to keep up to date with taxonomic revisions in all microalgal classes and hence outdated nomenclature is in some cases used. The fact that coccolithophores exhibit dimorphic haplo-diploid life cycles with each stage historically having been given a different taxonomic designation, as well as the recent realization that cryptic or pseudo-cryptic speciation may be a common phenomenon in the coccolithophores (see contributions to this volume by Geisen et al., Quinn et al. and de Vargas et al.) may complicate matters further. Examples exist in the literature of studies which have drawn conclusions based on the results of culture experiments using strains which we believe (following subsequent acquisition) to have been mis-identified, and also of the use of names invalidated decades prior to the actual studies.

Future exploitation of the existing culture resource

A recurrent theme through the review of the history of research involving cultured coccolithophores was the focus on two species, *E. huxleyi* and *P. carterae*. The latter species has become a model for studies of the calcification process partly by chance, although the fact that it is easy to culture has certainly been a contributing factor. The reasons for the focus on *E. huxleyi* are clear when satellite images of enormous white water blooms, generally considered to be dominated by this species, are seen. The relatively recent explosion in the volume of culture studies on *E. huxleyi* was triggered by such enhanced observational capacities which have served to highlight the abundance and biogeographic ubiquity of this species, together with increasing emphasis on global change studies and interest in the role of calcifying microorganisms and DMS producers in global biogeochemical cycles. Intensive culture investigations on *E. huxleyi* in the last decade mean that this coccolithophore must now rank among the best studied of all microalgal taxa, along with model diatom species such as *Skeletonema costatum*, and the freshwater chlorophyte *Chlamydomonas reinhardtii*. Despite outstanding progress, we are far from having a full understanding of the physiological and biochemical processes that contribute to the ecology and global impact of *E. huxleyi*, and a continued focus on this species in coming years is to be expected. The extraordinary environmental tolerances and robustness of this species mean that complex culture experiments can be planned with confidence.

Through the application of molecular DNA-based RAPD 'fingerprinting' techniques, Medlin et al. (1996) revealed considerable intraspecific genetic diversity

within *E. huxleyi*, even between clones isolated from the same bloom. Different *E. huxleyi* culture clones have been shown to differ in a number of physiological and biochemical traits, heterogeneity which has implications for all planning and interpretation of experimental work on *E. huxleyi* (Paasche 2002). The conclusion of Paasche (2002) that confusion in the literature can only be avoided if clone designation and origin is specified cannot be stressed too highly, and this of course applies not only to culture work on *E. huxleyi*, but to that on all coccolithophores.

The fact that the life cycle of *E. huxleyi* involves the alternation of a heterococcolith-bearing non-motile (C cell) phase and a non-calcifying flagellate (S cell) phase was first reported 30 years ago by Klaveness (1972). This author provided preliminary evidence that this cycle was haplo-diploid in nature, and this was later confirmed by Green et al. (1996) who quantified the DNA content of each phase using flow cytometry. Despite the possibility that the non-calcifying haploid S cell phase plays a critical role in the ecology of this species (Green et al. 1996), it has largely been ignored in both field and culture studies. In field studies, this is mainly due to the technical difficulties of observing and correctly identifying the S cells, but in culture studies the reasons are less clear; pure cultures of this phase have been available for some time, and, like the C cell phase, it is easy to culture. Despite the wealth of ecophysiological information available for the C cell and N cell phases (the latter being diploid, non calcifying, non-scale bearing, non-motile cells which are presumed to be C cells which have lost the capacity to calcify) in the life cycle of *E. huxleyi* (see Paasche 2002), there is very little comparative data for the S cell phase. The possession of flagella implies a different ecology for the S cell phase, and this may well be accompanied by physiological differentiation. In other words, there is as yet insufficient information to be able to generalize experimental results to different life cycle stages within a clone, let alone to different clones of a species. Awaiting experimental comparison of the ecophysiology of different life cycle stages, it is highly recommended that culture studies on *E. huxleyi* and on other coccolithophores, for which life cycle associations are known, should clearly specify which life cycle stage was employed.

The increasing body of knowledge on *E. huxleyi* has undoubtedly raised awareness of the global impact of coccolithophores, but has also fostered false perceptions. The terms *E. huxleyi* and coccolithophore are often used interchangeably by the non-specialist, insinuating either that *E. huxleyi* is the only important coccolithophore species in ecological terms, or that it can be taken as typical of all coccolithophores. In reality, of course, neither is the case. *E. huxleyi* is the most abundant and ubiquitous coccolithophore living in the contemporary ocean, often occurring at a relative abundance of 60–80%, but many other coccolithophore species may dominate communities in particular environments (see review of the biogeography of living coccolithophores by Winter et al. 1994). Taking carbonate flux calculations as an example of the potential importance of other coccolithophore species in global biogeochemical cycles, it should be noted that single coccoliths of larger species such as *Coccolithus pelagicus* contain up to 50 times more calcite than those of *E. huxleyi* (Samtleben and Bickert 1990). From the limited amount of comparative ecophysiological data available (see review by Brand 1994), as from analysis of the ecological distribution of coccolithophores (Young

1994), it is clear that *E. huxleyi* along with *P. carterae* are located at the upper (*r*-selected) limit of the *r*-*K* ecological gradient of coccolithophores, with ecophysiological adaptations geared towards maximizing growth rate. The majority of coccolithophore species, however, are likely to be located nearer the extreme lower (*K*-selected) limit of this gradient, implying very different physiological adaptations honed for optimizing the efficient use of limiting resources. In many respects *E. huxleyi* clearly cannot be considered as a typical coccolithophore, a view first put forward over half a century ago by Braarud and Fagerland (1946), who predicted that "the behavior of *E. huxleyi* in culture may not prove to be characteristic of the coccolithophores as a whole".

Comparative culture studies involving a range of coccolithophore species should therefore be encouraged. Of the coccolithophores currently maintained in culture, several belong to placolith-bearing taxa which may dominate oceanic coccolithophore assemblages (*Gephyrocapsa*, *Coccolithus*, *Calcidiscus*, *Oolithotus*, *Umbilicosphaera*) and hence may be considered important in a global context. Of these species, only *G. oceanica* has been exploited in culture experiments on anything more than a sporadic basis, but being closely related to *E. huxleyi*, physiological and biochemical differences are likely to be the least pronounced. The heterococcolith-bearing stages of all of these coccolithophore species are amenable to laboratory culture using standard methods and culture media including artificial seawater-based media (see below), and there are therefore no obvious major technical barriers to the application of the type of experimental design previously used for *E. huxleyi* to these species. Axenicisation of cultures of these and other coccolithophore species is one obvious priority which would significantly broaden the scope for their incorporation in physiological and biochemical studies.

The prospects for including the motile oceanic coccolithophores currently in culture (including *S. pulchra*, *C. mediterranea*, *H. carteri*, *A. robusta* and the holococcolithophores) in laboratory experiments are currently somewhat limited since they tend to be less resistant to mechanical stress and do not grow well in currently available artificial seawater-based culture media (see below). The former problem means that it is unlikely that continuous culture techniques will be applicable to these coccolithophores. Carefully designed semi-continuous culture experiments should, however, prove possible (see, for example, the methods designed for the semi-continuous culture of dinoflagellates by McIntyre et al. 1997). Artificial seawater media are particularly useful for investigations into nutrient physiology since they are almost completely defined from a chemical point of view. At present the best compromise solution for such studies on these motile coccolithophores is the use of clean oceanic seawater as the base for culture media. The eventual refinement of existing natural seawater- and artificial seawater-based culture media is essential for increasing the scope of culture experiments using all coccolithophores currently in culture. Such research is potentially very time consuming and outwardly in attractive in the sense that in general it will not provide any quick answers to the current key questions in coccolithophore research. There is, however, a fundamental need to develop our understanding of the biodiversity of this group which in the long term can only be achieved by increasing the range of coccolithophores brought into culture (a key issue expanded

on in the following discussion), but in the immediate future would be greatly enhanced by a more complete exploitation of the culture resource which already exists.

The isolation and culture of coccolithophores

The isolation of any microalgal species into clonal laboratory culture provides the key resource which potentially enables an exponential increase in our understanding of the evolution, the biology and the interactions of the species with the environment. Coccolithophores are generally held to be particularly difficult to culture, a scenario which could restrict the development of knowledge of this group in the future, but is this really the case?

A comparison can be made between the number of species that have been cultured and the total number of (known) species, providing an indicative value of 'success rate' for culturing. According to our estimations: 30 out of a total of 149 heterococcolithophores (20.8%) have been cultured (since heterococcolithophores and holococcolithophores are linked in a haplo-diploid life cycle, the total number of heterococcolithophores may be the most accurate indicator of specific diversity available); 38 out of a total of 156 species (24.4%) have been cultured if members of the Isochrysidaceae, which can be considered to be coccolithophores which have lost the ability to calcify (see Sáez et al. this volume), are included; 43 out of a total of 229 (18.8%) known life cycle stages (heterococcolithophores, holococcolithophores and known non-calcifying stages) have been cultured. These calculations are obviously influenced by our current understanding of the biological species concept in coccolithophores and if, as seems increasingly likely, cryptic or pseudo-cryptic speciation is a phenomenon common across the coccolithophores (see de Vargas et al. this volume), the approximate figure of four out of every five coccolithophores which remain to be cultured may be considerably underestimated.

By contrast, the majority (>90%) of known non-coccolithophore haptophytes have been cultured. Taxonomic descriptions of microalgae which do not have a mineralized or resistant organic cell covering are, to a large extent, only possible with cultured material. Of the non-calcifying haptophytes, only certain distinctive members of the genus *Chrysochromulina* have been described without ever having been cultured. In contrast to the situation in the coccolithophores, it is likely that many non-calcifying haptophyte morphospecies remain to be described (for example, the OLI clades in the phylogenetic tree presented by Sáez et al. this volume). For the known haptophytes as a whole, the success rate of culturing is therefore currently around 40% (98 of 236 species).

These figures for coccolithophores and haptophytes as a whole compare favorably with the success rates of culturing in the other two main marine phytoplankton groups, the diatoms and the dinoflagellates. The main international culture collections listed in Table 2 hold approximately 200 species of marine diatoms and 180 species of marine dinoflagellates. According to the estimations of

Sournia (1995), there are approximately 1300 described species of marine diatoms and 1700 described species of marine dinoflagellates, and the culturing success rates for these groups can thus be estimated at 15.4% for the diatoms and 10.6% for the dinoflagellates. The actual total number of cultured species in these groups is, of course, likely to be significantly higher since numerous smaller, specialized culture collections exist in laboratories worldwide, but on the other hand species diversity in these groups is also suggested to be seriously underestimated (Andersen 1992).

In relative terms, coccolithophores do not appear, therefore, to be substantially more difficult to culture than other groups of microalgae. This conclusion, although in some respects potentially misleading, does serve to make the point that coccolithophores should not be automatically considered as especially difficult to bring into culture, a view which would be likely to discourage future attempts to isolate previously uncultured species. The fundamental point which should be retained from this analysis, however, is that the vast majority of species in most taxonomic groups of microalgae, including the coccolithophores, have never been successfully cultured. This fact has critical consequences for our understanding of microalgal biodiversity and marine ecosystem functioning, and the need to address this issue is one of the key challenges in marine biology today, although the difficulty in funding such specific research (and in funding culture collections in general) reflects the general lack of awareness of this problem among policy makers.

Over the years several excellent reviews of microalgal culturing techniques have been published (e.g. Provasoli et al. 1957; Provasoli and Pintner 1960; Stein 1973; Guillard 1975; Guillard and Keller 1984; Brand 1990). In general, the methods that have been used for culturing coccolithophores do not differ greatly from those typically used for other microalgae. We do not, therefore, intend to provide an exhaustive methodological guide (for which readers should refer to these previous reviews and the references therein), but rather a general overview of the principles and methods involved together with specific examples from the literature and from our own experience of how these have been applied to the culture of coccolithophores. The examples from the literature and the statistics given are drawn from a review of over 100 publications involving diverse aspects of the study of cultured coccolithophores. This list is clearly not inclusive, but the trends observed are believed to be representative and are certainly interesting and informative. For example, of all of the culture manipulations in this survey (involving 29 species in total), over three quarters employ one of three species: 53% involve *E. huxleyi*, 15% *P. carterae* and 8% *G. oceanica* (an exhaustive literature review would very probably accentuate the dominance of these species in culture studies).

Collection and transportation of samples

Finding microalgae is not hard... (Brand 1990). A rigorous enough search will reveal coccolithophores (at least cells of *E. huxleyi*) in a liter of water sampled just about anywhere in the surface waters of the world oceans. A wide variety of tech-

niques can be used for the initial collection of microalgal samples, and these can be broadly categorized into collection of unconcentrated or concentrated samples. Unconcentrated samples can be collected simply by dipping a clean PVC bottle or bucket into surface water (this, along with scraping of rocks, is the only practical method for collection of the littoral coccolithophore species), or can be collected at different depths using more sophisticated devices such as Niskin bottles. Ship-board pumps can also be used, but in our experience only the most robust species survive this collection method. Following sampling, ideally the microalgae in the samples should be exposed to as little stress as possible, but for practical purposes it is most often necessary to concentrate the microalgal cells in these samples prior to isolation. This can be achieved by filtration, either through membrane filters followed by resuspension in a small volume of seawater (all but the gentlest filtration is likely to damage the more fragile coccolithophores), or through Nitex sieves of 5 or 10 μm pore size for the larger species. Alternatively, non-motile coccolithophores can be concentrated by sedimentation, and at least some motile species and stages by application of a concentrated light source to one part of the sample container (the majority of motile coccolithophores currently maintained in culture are positively phototactic). The use of Nitex plankton nets allows the *in situ* processing of larger volumes of seawater and hence the collection of pre-concentrated samples. Again, however, the smaller and more delicate coccolithophore species are not likely to be present or intact in net samples. Following collection and concentration it is often advisable to filter the sample through a 50–100 μm metal sieve in order to remove the larger zooplankton grazer species, particularly if isolations cannot be conducted immediately.

In general it is very important to transport samples at or below the temperature of collection (usually a cool-box is necessary). During prolonged transportation (during scientific cruises for example) the sample bottles should receive some light, but not direct sunlight. For transport from port to laboratory or postage from laboratory to laboratory, sample bottles should be completely filled in order to minimize turbulent mixing. In our experience, customs officials around the world rarely understand the meaning of the term phytoplankton and even less so coccolithophore, and hence in order to avoid critical delays it is perhaps advisable, when possible, not to volunteer information about samples or at least to remain vague when questioned about their contents. Correct procedures for the disposal of algae in the laboratory should, of course, at all times be followed in order to prevent the introduction of 'alien' species to local waters.

Isolation techniques

...but growing them may be (Brand 1990). In general, the objective is the establishment of a genetically uniform (clonal) strain by the isolation of a single cell. There are several ways of isolating a single cell into culture (see reviews cited above for detailed descriptions of these methods). Micropipette isolation has proved the most successful method for isolating heterococcolithophores. This method is best conducted using either an inverted or a binocular dissecting micro-

scope. The higher magnification of the former has certain advantages (the isolator is more likely to be able to distinguish between algal cells, and smaller cells are more easily observed), but using a dissecting microscope generally allows a greater number of isolations in a shorter time. Since many coccolithophore species are susceptible to temperature fluctuations, we advocate the latter method, following the principle of isolating anything which vaguely resembles a coccolithophore and identifying and selecting those which subsequently grow. Alternatively, an ice block can be placed under the micro well plate in order to minimize overheating of the isolation medium during prolonged isolation sessions. Under the dissecting microscope coccolithophores can be distinguished by the (usually dark) halo which surrounds the cell (corresponding to the coccosphere) and for motile species and stages by their characteristically slow, gently rotating swimming motion. For reference purposes, higher magnification light microscope images of a number of coccolithophore species are available on the CODENET website http://www.nhm.ac.uk/hosted_sites/ina/CODENET. Since different coccolithophore species have different medium preferences (see below), it is advisable to use a variety of media and a range of medium concentrations (including unenriched) when isolating, where possible using sterilized seawater from the sampling location as the medium base.

The preferred method is to isolate single cells from the sample before any enrichment, thus avoiding the problem of selecting preferentially for those species or genotypes that grow most rapidly under the prolonged sampling or enrichment procedure used (Brand 1990). Even when this is possible, it may prove useful to enrich a series of sub-samples with a variety of (improvised) medium enrichments, with and without germanic dioxide (an inhibitor of diatom growth); often species which were at very low abundance or were undetected in the original sample become more abundant, increasing the chance of successful isolation.

Of the other methods which have been successfully employed for coccolithophores (either in the literature or from our experience), serial-dilution culture may be particularly suitable for smaller and more delicate species, but this method is very time-consuming, produces cultures primarily of the dominant species which can usually be isolated by standard methods, and the clonality of the cultures cannot be guaranteed. Isolation from streaked agar plates has been used for the in-shore and littoral coccolithophores (see below), which, however, are generally not difficult to isolate by micropipette.

With a little innovation, it is entirely possible to reduce the volume of equipment needed for the isolation and culture of phytoplankton to a portable size and to conduct isolations on-board scientific vessels or in other non-laboratory locations.

Culture media

Media for the culture of marine phytoplankton consist of a seawater base (natural or artificial) typically supplemented by various nutrients essential for microalgal growth.

Seawater base

The quality of water used in media preparation is very important. Natural seawater can be collected near-shore, but its salinity and quality is often variable and unpredictable (due for example to anthropogenic pollution or toxic metabolites released by algal blooms in coastal waters). The quality of coastal water may be improved by ageing for a few months (allowing bacterial degradation of inhibitory substances), by autoclaving (heat may denature inhibitory substances), or by filtering through acid-washed charcoal (which absorbs toxic organic compounds). Most coastal waters contain significant quantities of inorganic and organic particulate matter, and therefore must be filtered before use (e.g. Whatman no. 1 filter paper). The low biomass and continual depletion of many trace elements from the surface waters of the open ocean by biogeochemical processes makes this water much cleaner, and therefore preferable for culturing purposes, but it is often difficult and expensive to procure. All coccolithophore species in the CODENET collection are maintained in aged, filter-sterilized seawater collected in the Baie de Seine off the Normandy coast, and are thus clearly able to grow in what must be considered low quality seawater. The coccolithophores cultured to date are for the most part those found in eutrophic waters, and oligotrophic adapted species are likely to be much more sensitive to water quality. In all cases certain precautions should be taken, such as the collection of seawater in clean polyethylene carboys from the front of the boat (or pump from subsurface), in order to minimize the risk of contaminating the seawater base.

The literature survey reveals that 94% of culture manipulations of coccolithophores have been conducted in media with a natural seawater base. The alternative, artificial seawater made by mixing various salts with deionised water, has the advantage of being well-defined from the chemical point of view, but they are very laborious to prepare and often do not support satisfactory algal growth. Trace contaminants in the salts used are at rather high concentrations in artificial seawater because so much salt must be added to achieve the salinity of full strength seawater, and hence it is essential to utilize reagent grade chemicals (see Berges et al. 2001). Among the more successful artificial seawater media that have been developed are the ESAW medium of Harrison et al. (1980) with modifications outlined by Berges et al. (2001), and the AK medium of Keller et al. (1987). In the literature only three coccolithophore species, *E. huxleyi*, *G. oceanica* and *P. carterae*, have been cultured in artificial seawater media. A wide variety of artificial seawater recipes have, however, reportedly been used. In our trials (unpublished results), the diploid heterococcolith-bearing stages of several of the coastal coccolithophore species (Pleurochrysidaceae and Hymenomonadaceae) as well as members of the Noelaerhabdaceae and the Coccolithaceae which are maintained in the CODENET collection showed acceptable growth in artificial seawater (at best equivalent to growth in natural seawater media), ESAW proving the most suitable and aquarium salt mixes the least suitable of the recipes tested. The non-calcifying flagellate phase of *E. huxleyi* was the only motile coccolithophore other than the coastal species which was able to grow in ESAW medium, albeit at a reduced rate relative to natural seawater medium. Artificial seawater media are particularly

useful (and in some cases essential) for investigations of the nutrient physiology of microalgae, but further manipulations of the available recipes are clearly required before studies of this kind can be conducted on a representative selection from across the phylogenetic diversity of cultured coccolithophores.

Medium supplements

Since the first simple culture medium recipes of the early nineteenth century, there has been a general trend towards the use of lower nutrient concentrations and more well-defined (often synonymous with more complex) and reproducible culture media as the specific nutrients needed became known (Brand 1990). As a result, a wide range of culture medium recipes can be found in the literature, and in addition many workers have tended to make minor adjustments to these recipes to suit the requirements of their algae of interest or sometimes according to a personal whim. In our survey, three medium recipes (or dilutions thereof) accounted for nearly two thirds of culture manipulations with coccolithophores. The most commonly employed medium supplement (40%) is f/2 (Guillard 1975), followed by K (Keller et al. 1987) with 17% and Erdschreiber with 6%. The latter is the only one of the three to include soil extract and, for reasons outlined below, this medium recipe has rarely been employed in recent times. In practice, dilutions of f/2 and K medium (e.g. 10%, equivalent to f/20 or K/10) are sufficient to maintain good growth of the majority of coccolithophores cultured to date. The recipes of these and a variety of alternative marine culture media are available on the WWW pages of the major culture collections (see Table 2 for URL addresses).

It was first discovered that soil extracts were beneficial to the growth of many marine phytoplankton in the 1920s. It is now known that soil extract provides various trace elements and vitamins essential for algal growth, metal complexing organic compounds that sequester potentially toxic metals, and organic compounds which keep iron in solution and allow for its photoreduction. The disadvantage of using soil extract lies in its unquantified and highly variable composition. As the functions of different components of soil extract have been identified, it has been replaced by specific compounds. In our experience the addition of soil extract to the culture medium is beneficial to the growth of all haploid (motile non-calcifying or holococcolith-bearing) stages of coccolithophore species in the CODENET collection, as well as to the diploid heterococcolith-bearing stages of the two members of the Syracosphaeraceae, *S. pulchra* and *C. mediterranea*, that are in culture. Soil extract is routinely filtered through 1 μm filter paper and autoclaved, but when an additional filter-sterilization step is employed using 0.2 μm membrane filters its beneficial effects to the growth of these species and stages are reduced (Houdan, unpublished data). It seems likely that the retention of particulate organic matter and possibly dissolved organic molecules on the membrane filter is responsible for this difference; phagotrophy has been reported in several non-calcifying haptophytes as well as the haploid stage of *C. braarudii* (Parke and Adams 1960), and some coccolithophores may be capable of utilizing dissolved organic molecules (see Brand 1994). For diploid stages of the coastal coccolithophores the addition of soil extract is neutral, whereas for the diploid stages of all

other species maintained it has a negative impact on growth (Houdan and Probert, unpublished results). While sometimes useful for isolation and routine maintenance purposes, soil extract should only be used on a non-experimental basis.

Most coccolithophores that have been cultured are autotrophic (or more accurately auxotrophic) and can utilize inorganic forms of macronutrients. Nitrate is the nitrogen source most often used in culture media, but may be toxic to oceanic species, potentially even at a fraction of the high concentrations used in most culture media. Ammonium is the energetically preferential form of nitrogen for many algae since it does not have to be reduced prior to amino acid synthesis, the point of primary intracellular nitrogen assimilation into the organic linkage. For cultured *E. huxleyi*, several recent studies (Flynn et al. 1999; Page et al. 1999; Varela and Harrison 1999) have shown a 50% depression of nitrate uptake rates by ammonium concentrations in the region of 0.2–0.5 μM . It is well known that ammonia can be toxic at high concentrations because of its high permeability through the cell membrane, and Brand (1986) recommended the use of 10 μM or less, concentrations which can generate easily detectable biomass levels, in attempting to culture oceanic phytoplankton.

Organic nitrogen compounds that can be used by at least some *E. huxleyi* clones include a few amino acids (Ietswaart et al. 1994) and low molecular weight amides and, in the presence of nickel ions, also urea and purines such as hypoxanthine (Palenik and Henson 1997). Many microalgae are also capable of using a variety of dissolved organic carbon compounds (Droop 1974), among these coccolithophores is the littoral genus *Pleurochrysis* (see Paasche 1968). Another group of organic compounds known to be required by many phytoplankton are the vitamins. Roughly half of all microalgal species tested have been shown to have a requirement for vitamin B₁₂, which appears to be important in transferring methyl groups and methylating toxic elements such as arsenic, mercury, tin, thallium, platinum, gold, and tellurium, around 20% need thiamine, and less than 5% need biotin, while no other vitamins have ever been demonstrated to be required by any photosynthetic microalgae (Brand 1986). *E. huxleyi* requires thiamine, but not vitamin B₁₂ (Carlucci and Bowes 1970), but with the lack of comparative data for other coccolithophores it is probably prudent to systematically add these three vitamins to culture media.

The main problem with the use of dissolved organic compounds in culture media is that bacteria, with a high surface area to volume ratio relative to microphytoplankton, are superior competitors when low concentrations of these substances are employed. This would suggest the need to take steps to axenicise the cultures, which may be problematic for delicate oceanic species. It is also known, however, that the growth of some microalgae is stimulated by the presence of bacteria, possibly due to the secretion by bacteria of substances, for example photosensitive vitamins (Brand 1986), which are needed by the phytoplankton but not present in the media. In addition, inobligate phagotrophy is probably widespread in the haptophytes (see Billard and Inouye this volume) and bacteria may thus represent a potential food source. Until more is known about the potential role of organic nutrients in coccolithophore nutrition, it is advisable to try out different compounds and methods simultaneously when isolating new coccolithophores.

Inorganic (ortho) phosphate, the phosphorus form preferentially used by microalgae, is most often added to culture media, but organic (glycero) phosphate is sometimes used, particularly when precipitation of phosphate is anticipated (when nutrients are autoclaved in the culture media rather than separately, for example). In culture experiments, *E. huxleyi* has been demonstrated to have an extraordinarily high affinity for orthophosphate, and phosphorus-limited cells subjected to an external phosphate pulse were shown to accumulate surplus phosphate in amounts up to seven times greater than the steady state phosphorus cell quota (Riegman et al. 2000). Furthermore, the presence of cell surface constitutive as well as inducible alkaline phosphatases enables *E. huxleyi* to use organic phosphate esters at the nanomolar concentration level (Riegman et al. 2000). There is no comparative data for phosphate uptake and storage in other coccolithophores, but the use of inorganic phosphate should theoretically be suitable for the culture of oceanic species.

The trace metals which are essential for microalgal growth are incorporated into essential organic molecules, particularly a variety of coenzyme factors which enter into photosynthetic reactions, and hence they exert important controls on growth limitation by the major algal resources – light, inorganic carbon and nitrogen (see review by Sunda 2000). Of these metals, the free ionic concentrations of iron, zinc, manganese, cobalt, copper, cadmium, selenium and possibly certain other elements in natural waters may influence microalgal growth, either as nutrients or as toxins. The relationships between chemical speciation of metals and biological availability are complex. Chelators act as trace metal buffers, maintaining constant concentrations of free ionic metal. Without proper chelation some metals (such as copper) are often present at toxic concentrations, and others (such as iron) tend to precipitate and become unavailable to phytoplankton. In natural seawater, dissolved organic molecules (generally present at concentrations of 1–10 mg l⁻¹) act as chelators. The most widely used chelator in culture media additions is ethylenediaminetetraacetic acid (EDTA), which must be present at high concentrations since most complexes with Ca and Mg, present in large amounts in seawater. High concentrations have, however, occasionally been reported to be toxic to microalgae. As an alternative the organic chelator citrate is sometimes utilized, having the advantage of being less influenced by Ca and Mg. The molar ratio of chelator : metal in culture media ranges from 1:1 in f/2 to 10:1 in K medium. High ratios may result in metal deficiencies for coastal phytoplankton (i.e. too much metal is complexed), but are suitable for oceanic species for reasons outlined below. The inorganic complexation (and hence the free ionic concentrations) of trace metals in culture media can be calculated through the use of thermodynamic models (see Twiss et al. 2001).

Algal species isolated from oceanic environments, where trace metal concentrations are very low, are able to grow at much lower concentrations of ionic iron, zinc and manganese than species isolated from coastal environments. Both the iron (Brand 1991; Muggli and Harrison 1996, 1997) and zinc (Sunda and Huntsman 1992, 1995) requirements of *E. huxleyi* are low relative to coastal diatoms. The coccolithophores *E. huxleyi*, *G. oceanica*, *C. leptoporus*, *U. hultburtiana* and *U. sibogae* are all able to grow at much lower concentrations of iron, zinc and

manganese than the coastal species *P. carterae* (Brand et al. 1983). *E. huxleyi* produces iron-binding ligands (Boye and Van den Berg 2000) that may ensure availability of this metal in the natural environment. Cellular metabolic requirements for limiting metals can also be decreased by altering metabolic pathways or by changing the metalloenzyme content of key pathways; *E. huxleyi* has a primary requirement for zinc that can partially be replaced by cobalt (Sunda and Huntsman (1995). It is not known whether other coccolithophores have such adaptations, but in any case the lower growth rate of oceanic species partially solves the problem: low growth rates mean an increase in cellular concentrations at a given uptake rate (uptake rates in oceanic phytoplankton are thought to have already evolved to near their chemical and physical limits), decreasing the metabolic demand for metalloenzymes (Sunda 2000).

Using standard natural seawater-based culture medium recipes, trace metal limitation is therefore likely to be less of an issue than trace metal toxicity for the culture of oceanic species. The provision of manganese, zinc and cobalt in culture medium should not be problematical since even fairly high concentrations are not thought to be toxic to algae. High concentrations of iron could potentially inhibit phytoplankton growth (Brand 1986), although there is little experimental data to support this. Oceanic phytoplankton may be particularly sensitive to copper toxicity. *E. huxleyi* produces copper-complexing ligands (Leal et al. 1999) and is adapted to tolerate much higher concentrations of copper than other species (Brand et al. 1983). Most algae either do not need copper, or need so little that free ionic copper concentrations of 10^{-19} M are sufficient to maintain maximum growth rates (Brand 1986). Cadmium is known to interfere with calcification and it has been found that coccolithophores are more sensitive to cadmium toxicity than other phytoplankton (Brand et al. 1983). A reasonable approach to developing culture media for the culture of oceanic coccolithophores would therefore be to lower concentrations of trace metals (particularly copper and cadmium) relative to standard media and also to minimize concentrations of chelators by calculating the exact amount necessary to maintain the desired free ionic metal concentrations. In order to avoid unwanted trace metal contamination, oceanic seawater (or ultra-clean bi-distilled water for artificial media) and highest quality reagent grade chemicals are also highly recommended.

Agar media

Some marine microalgae grow well on solid agar substrate. Of the coccolithophores, the inshore coastal species (Pleurochrysidaceae, Hymenomonadaceae and *Cruciplacolithus neohelis*) are particularly suited to this method of culture, which is effective for long-term maintenance of these species. In our experience, certain of the non-motile placolith-bearing coccolithophores may be maintained for short periods on agar medium and this may be useful for cleaning cultures infected with bacteria (clean colonies being isolated from the plate into fresh liquid medium).

Sterilization of culture media

Certain coccolithophore species and life cycle stages (particularly those which are motile and possess a haptonema) do not grow well in autoclaved media due to one or a combination of a number of potential factors. The high temperature and pressure during autoclaving result in significant chemical changes to seawater including pH increases (as CO₂ is driven out of solution), the formation of precipitates and the denaturation of organic compounds. Certain measures can be taken to alleviate or minimize the first two of these factors (lowering of pH by addition of HCl prior to autoclaving, the addition of buffers such as bicarbonate or TRIS, rapid cooling after autoclaving, the use of Teflon bottles during autoclaving, separately autoclaving the seawater base and medium enrichments), but in our experience these species show poor growth even when these precautions are taken. Motile microalgae are known to be particularly sensitive to precipitates in the medium (e.g. Keller et al. 1987), and as discussed above many of the motile coccolithophores may be mixotrophic and hence benefit from the presence of dissolved organic molecules in the medium, two possible reasons to account for inhibition of growth in autoclaved media. These problems can be avoided if media are filter-sterilized using 0.2 µm membrane filters, but it must be remembered that viruses will pass through such filters unaffected and that given filter pore sizes are an average, not an absolute value, and therefore that some pores in these filters may be substantially larger than 0.2 µm. For the routine maintenance of the CODENET collection a compromise is employed: a combination of tyndallisation (heating of the seawater to 75°C which should kill most organisms which pass through the pre-filtration, but should limit chemical changes to the seawater) followed by filter-sterilization. For experimental purposes, the use of artificial or autoclaved natural seawater base media is often preferable.

Types of culture vessel

Culture vessels should have the following properties: non toxic (chemically inert); reasonably transparent to light, easily cleaned and sterilized, provide a large surface to volume ratio. Coccolithophores can be cultured in Erlenmeyer flasks or tubes (glass or polycarbonate) of various sizes with cotton, glass, polypropylene, or metal covers, or in polystyrene tissue culture flasks, purchased as single-use sterile units (e.g. Iwaki, Nunc, Corning). The latter have the advantage of allowing rapid observation of the state of the culture under a binocular microscope without opening the culture vessel, and although expensive, do not require technician time for cleaning and sterilization. An anecdotal observation is that coccolithophore cultures tend to grow more slowly and reach higher cell densities in glass Erlenmeyer flasks, making these suitable for long-term routine maintenance, particularly of robust species.

Pure, axenic cultures

If biological contaminants of eukaryotic nature appear in a culture, the best solution is to reisolate a single cell from the culture with a micropipette, and try to establish a new, clean clonal culture. For some coccolithophore species, the culture can be streaked on an agar plate in the hope of attaining a colony free of contaminants. These methods are useful for minimizing bacterial contaminants, but a truly axenic culture cannot be ensured. To achieve this, antibiotics must be used. The treatment of an actively growing culture with a mixture of penicillin, streptomycin and gentamycin (each at concentrations of 50–500 mg l⁻¹) for around 24 hours followed by micropipette isolation or agar streaking is generally recommended. Other antibiotics that can be used include chloramphenicol and tetracyclin. Other than for strains of the diploid phase of *E. huxleyi* and *G. oceanica*, axenic coccolithophore cultures have rarely been reported. We suspect that axenicisation of most coccolithophore species maintained in culture and particularly of motile stages will prove problematic since these may have obligate relationships with bacteria. It should also be remembered that in reality there is no way of demonstrating that a microalgal culture is completely axenic. In practice, therefore, axenic usually means ‘without demonstrable unwanted prokaryotes or eukaryotes’.

Environmental parameters

Temperature

The temperature at which cultures are isolated and initially maintained should ideally be as close as possible to the temperature at which the organisms were collected. All microalgae have an optimum temperature for growth, above which growth rates typically rapidly decline with small temperature increments; temperatures lower than the optimum for a species are better supported and should be used for routine culture maintenance. The temperature tolerances of cultured coccolithophores are reflected in their biogeographic ranges. Brand (1994) reported that of cultured coccolithophores, the coastal species *P. carterae* has the widest temperature tolerance, followed by the cosmopolitan species *E. huxleyi* and then by the warm water species *G. oceanica*, *C. leptoporus*, *U. sibogae*, *S. pulchra*, the tropical oceanic species *Scyphosphaera apsteinii* having the narrowest temperature tolerance. We have recorded a similar trend, and of note is the fact that haploid stages typically have slightly higher temperature optima for growth than their diploid counterparts (Houdan and Probert, unpublished data). For all species maintained in the CODENET collection, a compromise temperature of 17°C is employed for routine maintenance. The literature review reveals that the majority of coccolithophore culture manipulations have been conducted at a temperature between 15 and 20°C (62%), with only 14% conducted above 20°C.

Light

Haptophytes have a photosynthetic pigment composition basically similar to that of diatoms and chrysophytes (see Van Lenning et al. @ this volume). The levels of light required to keep coccolithophores alive and to saturate growth and photosynthetic rates do not appear to be significantly different from those of other eukaryotic phytoplankton, while species living in eutrophic waters appear to need more light than those living in oligotrophic waters (Brand and Guillard 1981). Light intensities between 0.2–50% of full daylight ($1660 \mu\text{E s}^{-1} \text{m}^{-2}$) can be used for microalgal cultures, with 5–10% (c. $80\text{--}160 \mu\text{E s}^{-1} \text{m}^{-2}$) most often employed. Diploid stage cells of *E. huxleyi* do not show photoinhibition even at light intensities around $1000 \mu\text{E s}^{-1} \text{m}^{-2}$ (Nanninga and Tyrrell 1996). For the same species, culture in green and particularly blue light cause significant changes in pigment ratios relative to full spectrum light (Schlüter et al. 2000), but it is unclear whether this affects growth rates. Brand and Guillard (1981) demonstrated that the growth of oceanic coccolithophores (represented by *C. leptoporus*) is inhibited by long light photoperiods. *E. huxleyi*, *G. oceanica* and the coastal species can be grown in continuous light with little or no growth inhibition. In our survey of culture manipulations, 31% were conducted with a photoperiod of 12L:12D, 29% at 14L:10D, 20% at 16L:8D, 10% in continuous light and 10% in natural light (where photoperiod depends on location and season).

Salinity

A few species of coccolithophore live in freshwater or low salinity intertidal pools, but most are marine. Brand (1984) reported that, as expected, oceanic species tolerate only a narrow range of salinities and estuarine species can tolerate a much wider range. The CODENET collection, many strains of which were isolated from, and initially cultured in, high salinity Mediterranean waters (36–37.5ppt), is maintained in relatively low salinity (32–33ppt) English Channel water, seemingly with no adverse effects.

Mixing

Most small volume (up to 1 liter) coccolithophore cultures grow well without mixing, particularly when not too concentrated, but when possible, daily gentle manual swirling is recommended. Mixing of microalgal cultures may be necessary under certain circumstances, such as when using chemostat techniques or in concentrated cultures to prevent nutrient limitation effects due to stacking of cells and to increase gas diffusion. Mixing can be achieved by the use of a plankton wheel or roller table (about 1 rpm), by magnetic stirring or by bubbling with air. As for most microalgae, generally only the more robust (non-motile) coccolithophores are able to support physical disturbance; flagellate cells tend to aggregate into clumps and die, with the notable exception of the haploid stages of *E. huxleyi* and *G. oceanica* which grow well with air bubbling. The problem of CO_2 depletion in dense microalgal cultures may be reduced by having a large surface area of media

exposed to the atmosphere relative to the volume of the culture, or by bubbling with either air (CO₂ concentration 0.03%) or air with increased CO₂ concentrations (0.5 to 5%). The latter is generally not recommended for coccolithophores since it may lead to pH decreases and hence decalcification.

Chemostat cultures

Continuous culture techniques are particularly useful for physiological studies of microalgae. *E. huxleyi* and *G. oceanica* (in the diploid stages) are the only coccolithophore species which have been used in chemostat experiments. In practice, real continuous culture methods have rarely been applied, semi-continuous cultures, in which a volume of culture is replaced by fresh medium once per day, being the preferred technique. All other cultured coccolithophore species should theoretically be amenable to semi-continuous culture, but the relatively intense mixing that is required to maintain homogeneous cell density in continuous cultures is likely to limit the application of this technique to all but the most robust of non-motile stages.

Cryopreservation

Cryopreservation, the maintenance of living organisms in a frozen state, is a developing technique with enormous potential for the perpetual conservation of cultured cells without the continual turn-over of generations inherent to long-term culture maintenance using standard methods, and hence the avoidance of the problem of genetic drift. Historically, cryopreservation methods were developed principally for the conservation of bacteria, fungi, human and other animal tissues, but in recent years many of the main microalgal culture collections have initiated cryopreservation programmes. The CCMP (Bigelow, USA) reports the successful cryopreservation of strains of several coccolithophore species (*E. huxleyi*, *G. oceanica*, *P. carterae*, *P. roscoffensis*, *P. scherffellii*, *O. neapolitana* and *C. neohelis*) using 5–10% dimethyl sulfoxide (DMSO) as cryoprotectant, although apparently not all strains of each of these species have been successfully cryopreserved. Trials for the cryopreservation of these and other coccolithophore species in the CODENET collection are in progress.

Future prospects for culturing other coccolithophores

A breakdown of the taxonomic affiliations of the 36 coccolithophores cultured to date is given in Table 3. Of the species which have never been cultured, the heterococcolith-bearing stages of members of the Noelaerhabdaceae and the Coccolithaceae, together perhaps with members of the Pontosphaeraceae, can be considered to be the most likely to be cultured in the future using standard culture

methods. These coccolithophores are relatively robust (bearing placoliths or discoliths), probably non-motile, mostly have a wide biogeographic range (Winter et al. 1994) and tend to be more important in intermediate environments between extreme eutrophic and oligotrophic conditions (Young 1994). It can therefore be predicted that they should be able to tolerate the abnormal environment and elevated nutrient levels which are implicit with standard culture techniques. In the genus *Gephyrocapsa*, most members of which can be abundant in certain environments, only *G. oceanica* has been cultured. The other species in this genus are typically of such an extremely small size ($<3\ \mu\text{m}$) that it is probably a technical limit of isolation methods rather than the culture conditions themselves that has prevented their having been brought into culture until now.

The three families Syracosphaeraceae, Rhabdosphaeraceae and Calyptrosphaeraceae (holococcolithophores) contain the majority of the remaining coccolithophores which have never been cultured. These species (or life cycle stages) rarely dominate assemblages and hence do not have clearly definable biogeographic distributions, being found in upper and middle photic zone layers typically in oligotrophic, but also in intermediate environments. Of the other families, the Papposphaeraceae, which were until recently thought to be present only in polar waters (e.g. Thomsen et al. 1991) but have recently also been described from the Mediterranean Sea (Cros and Fortuño 2002) contain the most species. Certain of the species *incertae sedis* are globally distributed in particular environments: the umbelliform coccolithophores *Umbellosphaera tenuis* and *U. irregularis* (along with the Rhabdosphaeraceae *Discosphaera tubifera*) may dominate extreme oligotrophic assemblages, and the floriform coccolithophores *Florisphaera profunda* and *Thorasphaera flabellata* are the characteristic species of deep photic zone (150–200 m) assemblages in low to mid latitudes (Young 1994).

Table 3. Family-level taxonomic distribution of previously cultured coccolithophores (taxonomy from Jordan and Green 1994).

Coccolithophore Family	Species cultured / Total species diversity
Pleurochrysidaceae	7 / 7
Hymenomonadaceae	6 / 6
Noelaerhabdaceae	3 / 12
Coccolithaceae	9 / 17
Syracosphaeraceae	1 / 43
Rhabdosphaeraceae	1 / 18
Pontosphaeraceae	1 / 7
Helicosphaeraceae	2 / 4
Braarudosphaeraceae	0 / 2
Calciosoleniaceae	0 / 3
Ceratolithaceae	0 / 2
Papposphaeraceae	0 / 14
Calyptrosphaeraceae	6 / 55
Species incertae sedis	0 / 14

Although this group of coccolithophores has diverse ecologies, they all tend to be found most often in oceanic waters. This implies that they lie towards the *K*-selected end of the *r-K* ecological gradient (Brand 1994; Young 1994), and this is the factor most often stated to be responsible for the fact that they have not been cultured, for reasons associated with potential limitations of isolation and culture methods discussed below. Oceanic waters are also, however, relatively inaccessible, and this raises the possibility (or even probability) that no one has actually ever tried to isolate and culture the majority of these coccolithophores. This is of course difficult to assess with any certainty since unsuccessful isolations are rarely reported, but a logical first step would be to attempt to isolate and culture these species using current methods. *K* medium (or dilutions and derivations thereof), which was specifically designed for the culture of oceanic ultraphytoplankton, is perhaps the best available option for potentially culturing these oceanic coccolithophores, although a variety of medium recipes should be tried.

The oceanic environment is typically more stable (and therefore more predictable) than the eutrophic coastal environment, and in adapting to their environment oceanic species have evolved narrow environmental tolerances (Brand 1994). Physical environmental parameters (temperature, light, salinity) can be tightly controlled in culture and with special care also during the collection and isolation stages. The physical disturbance to cells, which is inevitable during collection and isolation, may be a problem for the most delicate coccolithophores (for example *Michelsarsia* and *Calciopappus* which have long leg-like appendages consisting of joined coccoliths) and here also some innovation will clearly be required. Problems with restraining and recreating the physical environment may be particularly important for culturing the deep photic-zone species; since the nutricline and thermocline usually coincide these species should be relatively insensitive to high nutrient levels, but adaptation to this unique environment may mean that they are particularly sensitive to increased light intensity (even to extremely low levels which would make collection and isolation problematic) and possibly also to decreased pressure. Until isolation of these species is attempted, these questions will remain unanswered.

For the majority of uncultured coccolithophores, however, considerations of the physical environment are likely to be secondary to the need to develop a chemical environment which is suitable for the growth of these oceanic species. One factor that these coccolithophore species have in common is that they are never very abundant. Even the umbelliform coccolithophores which may dominate oligotrophic environments rarely exceed 10^4 cells l^{-1} , whereas placolith-dominated assemblages in eutrophic conditions may regularly attain 10^7 cells l^{-1} (Young 1994). These oceanic species apparently have slow growth rates and do not respond rapidly to nutrient enrichment (e.g. Hulburt and Corwin 1969), but rather survive (and occasionally dominate) by virtue of their competitive abilities. There are two main factors relative to the nutritional requirements of these oceanic species which must be taken into account in order to develop more suitable culture media. Firstly, it is possible that the high concentrations of inorganic nutrients usually added to culture media will actually be toxic to oligotrophic adapted species. Brand (1986) argued that since oceanic phytoplankton rarely, if ever, experience

elevated nutrient levels, there are obvious evolutionary grounds to suggest that they may not have evolved (or have lost) adequate feedback mechanisms for tolerating extremely high substrate concentrations. In this context, a particular problem is that single isolated cells are susceptible to the preferential partitioning into the cell's protoplasm of various toxic substances present in the relatively large volume of culture media. Eutrophic-adapted species are more likely than oceanic species to possess the metabolic mechanisms required to cope with such a large and sudden influx of toxic substances. Secondly, it has been hypothesized that the utilization of organic nutrients becomes more important in oceanic waters (e.g. Jackson and Williams 1985; Amon and Benner 1994).

The problem with developing new culture media, as pointed out by Brand (1986), is that in order to culture a species we need to know its environmental requirements, but in order to develop a clear idea of its environmental requirements we need to study it in culture. Most of the coccolithophores that are in culture have thus far been under-exploited in terms of autecological and physiological investigations, and hence at present the information available for *E. huxleyi* provides the main base from which extrapolations, bearing in mind the two factors indicated above, must be made.

To conclude this discussion on the prospects for bringing a wider range of coccolithophores into culture it is worth highlighting that, in theory at least, there is no such thing as an unculturable microalga: if a species has not yet been cultivated it is either because no one has tried, or because current methods and technologies are not suitable for its culture. Improvements to current techniques can and will be made, although it is difficult to predict at what rate this will occur. It must be remembered that in culturing in general there are (within limits) no right and wrong methods; culture methods have only been developed by culturers using trial and error (usually based on theoretical considerations), and hence innovation is actively encouraged. If concerted efforts were to be made, taking into account the potential solutions to the (potential) problems of culturing oceanic species suggested and discussed above, we suggest that the majority of coccolithophore species could be successfully cultured within the next decade.

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A review of the phylogeny of the Haptophyta

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Summary

Most haptophytes are unicellular, photosynthetic flagellates, although some have coccoid, colonial, amoeboid or filamentous stages. Nearly all have a characteristic filamentous appendage, the haptonema, arising between the two flagella. We have amassed small subunit rRNA gene sequences (18S rDNA) from 125 haptophytes and aligned the sequences with those of over 300 published and unpublished chlorophyll *a+c* algae. Phylogenies were constructed using Bayesian, minimum evolution and weighted maximum parsimony analyses. The high divergence (6%) between members of *Pavlova* and the remaining haptophytes supports the division of the Haptophyta into two classes: the Prymnesiophyceae and the Pavlovophyceae (Edvardsen et al. 2000). Four major clades within the Prymnesiophyceae were identified that correspond to known taxa: one clade embraces Phaeocystales; the second includes members of the Prymnesiales; the third represents the Isochrysidales; and the fourth the Coccolithales. Two other minor clades contain taxa whose sequences were derived from a gene clone library. In the absence of information on cell morphology associated with these sequences we are unable to determine whether they belong to existing orders or if new orders should be erected. These taxa are not strongly related to any of the known cultured taxa. One to two per cent divergence in the 18S rRNA gene analysis warrants a separation above the level of family.

Introduction

The Haptophyta are a major lineage of chlorophyll a+c algae. The majority of known haptophytes occur as marine coastal or open oceanic planktic forms (Hibberd 1980; Green and Jordan 1994; Thomsen et al. 1994), although a few species thrive in freshwater. Many can form massive blooms (Birkenes and Braarud 1952; Berge 1962; Dahl et al. 1989; Blackburn and Cresswell 1993; Brown and Yoder 1994; Van der Wal et al. 1995; Lancelot et al. 1998), in some cases harming natural biota and commercial fisheries (Moestrup 1994; Edvardsen and Paasche 1998).

The haptophytes range in size from 2 μm (the upper limit of picoplankton) to macroscopic colonies (*Phaeocystis*; Thomsen 1986), and may occur as non-motile single cells (many coccolithophores), non-motile colonies of single cells embedded in mucilage (*Phaeocystis*), as motile single cells (most non-coccolithophores, e.g. *Chrysochromulina*, many coccolithophores e.g. *Syracosphaera*), or colonial flagellates (*Corymbellus*). Several haptophyte species form benthic filaments, and some may have amoeboid stages in their life cycle (Hibberd 1980). Many have alternate morphologically distinct forms, e.g., *Isochrysis galbana* (Parke 1949), *Phaeocystis globosa* (as *P. pouchetii* in Parke et al. 1971), *Chrysochromulina polylepis* (Edvardsen and Paasche 1992), and many coccolithophores (see Gayral and Fresnel 1983; Thomsen et al. 1991; Billard 1994; Billard and Inouye this volume). Most of these alternate morphotypes have been hypothesized to be alternate stages in a haplo-diploid life cycle (Billard 1994; Green et al. 1996; Cros et al. 2000; Geisen et al. 2002; Billard and Inouye this volume).

The haptonema, a filiform appendage situated between the two flagella, is the characteristic structural feature of most haptophytes. It can be very long, up to 160 μm in *Chrysochromulina camella*, with the ability to coil and uncoil (Leadbeater and Manton 1969), or may be short and flexible, or reduced to a few microtubules inside the cell, or (rarely) absent. The haptonema is used for attachment or in food capture (Inouye and Kawachi 1994).

The haptophytes contain one or two chloroplasts, each with an immersed or bulging pyrenoid. The nucleus is usually situated towards the center and antapical end of the cell, and the outer nuclear membrane is continuous with the CER, i.e., chloroplast endoplasmic reticulum. Haptophytes also have an endoplasmic reticulum, the peripheral ER, lying just beneath the plasmalemma (Jordan et al. 1995). The peripheral ER extends into the haptonema and is absent only in the area immediately around the flagella. In scale-bearing species scales are produced in the Golgi body (Manton and Parke 1962; Jordan et al. 1995), which is often arranged in a fan-like structure perpendicular to the long axis of the cell. Heterococcolith calcification occurs in Golgi-derived vesicles, holococcolith calcification occurs on Golgi-derived scales but appears to occur extracellularly (Young et al. 1999).

The standard classification of modern haptophytes was established by Parke and Green (in Parke and Dixon 1976), who recognized four orders: Coccosphaerales (coccolith-bearing), Prymnesiales (non-coccolith-bearing haptophytes, haptonema well developed), Isochrysidales (haptonema diminutive, including some

coccolith-bearing genera), and Pavlovales (with flagella of unequal lengths, the longer flagellum with hairs and scales, haptonema diminutive). Green and Jordan (1994), however, argued that only the Pavlovales were an unambiguously well-differentiated group and recommended subsuming the other three orders into the single subclass Prymnesiophycidae, order Prymnesiales.

Young and Bown (1997a, b) and Bown and Young (1997) provided a comprehensive classification of extant and fossil coccolithophores including 46 families (2 new). They considered that following Green and Jordan (1994) and including all these in the single order Prymnesiales would inhibit description of affinities and so used 12 orders (three new), including six with extant members, but with a clear understanding that the number of orders could be reduced as new information on relationships became available. A slightly revised version of this classification was used by Cros and Fortuño (2002) in their monograph of extant Mediterranean coccolithophores.

Edwardsen et al. (2000) and Fujiwara et al. (2001) published recent phylogenies of the Haptophyta, based on new molecular data and a review of cytological characters. Edwardsen et al. (2000) raised Pavlovales and Prymnesiales to class level (classes Pavlovophyceae and Prymnesiophyceae) because the 6% differences in the 18S rRNA gene between Pavlovales and Prymnesiales was consistent with the amount of differences found in other algal groups at the class level. At the ordinal level, roughly 1–2% differences are noted for the dinoflagellates (Saunders et al. 1997) and for the green algae (Friedl 1996). We find similar levels among the haptophyte orders.

The molecular data of Edwardsen et al. (2000) also allowed identification of four clades (or monophyletic groups, where all descendants belong to the group) within the Prymnesiophyceae at this level of differentiation, three rather closely corresponding to the traditional orders, Prymnesiales, Cocosphaerales, and Isochrysidales and a fourth including the numerous species of the distinctive genus *Phaeocystis*. Consequently they reinstated the traditional orders and introduced a new order, Phaeocystales. Their sample set did not include any members of the additional four orders of coccolithophores with extant members recognized by Young and Bown (1997b); the Syracosphaerales, Rhabdosphaerales, Zygodiscales and Stephanolithales. Fujiwara et al. (2001) did include *Helicosphaera carteri*, a member of Zygodiscales, in their phylogenetic analysis of *rbcL*, but they could not be conclusive about its phylogenetic position among coccolithophores.

In the class Prymnesiophyceae, the homodynamic or heterodynamic flagella are usually equal to sub-equal. The mature flagellum of several species has autofluorescence (Kawai and Inouye 1989). Members usually have organic, fundamentally plate-like scales, which may become complex (Leadbeater 1994 and references therein). In the coccolithophores, the scales of the outer layer are calcified and called coccoliths. The identification of haptophyte algae to species level relies heavily on scale and coccolith morphology. In the unmineralized genera, scale morphology has not been used as a taxonomic character above the species level, but in the coccolithophores, all taxon descriptors are dependent on coccolith morphology and structure (e.g., Deflandre 1952; Braarud et al. 1955; Heimdal 1993; Jordan and Kleijne 1994; Bown and Young 1997; Bown et al. this volume). A key

reason why this is possible and successful is that calcite, unlike cellulose or silica, is an anisotropic crystalline substance, the biomineralisation and shape regulation of which is a significant biochemical process. This both means that coccolith ultrastructure is more conservative and characteristic than is obvious from the superficial appearance of the coccoliths and that crystallographic orientation provides further key characters (Romein 1979; Young et al. 1992, 1999). One consequence of this is that the primarily paleontological classification based on cross-polarized light microscopy of isolated heterococcoliths has proven much more robust than might be expected, indeed in some cases more successful than biological classifications based on examination of whole coccospheres; separation of the Noëlaerhabdaceae from the Coccolithaceae was for instance made by paleontologists (Hay and Mohler 1967; Romein 1979) 20 years before it was accepted by biologists (Kleijne 1993; Jordan and Green 1994). Complex life cycles involving haploid and diploid generations have been hypothesized and documented for many of the coccolithophores as well as many *Chrysochromulina* species (Billard 1994; Green et al. 1996; Cros et al. 2000; Geisen et al. 2002; Billard and Inouye this volume).

In contrast to members of the Prymnesiophyceae, the Pavlovophyceae have two strongly anisokont (unequal length), heterodynamic flagella with a short haptonema. Autofluorescent flagella are unknown. The longer, anterior flagellum is often adorned with a covering of fine hairs and knob-like bodies, which may be either modified scales (Green 1980) or modified hairs (Cavalier-Smith 1994). Because plate-like body-scales are absent, species identification in the Pavlovophyceae is primarily based on the morphology of the knob-like bodies. Stigmata (eyespots) are found within the chloroplast in several species, but are often not associated with an overlying flagellum, as in some heterokont algae (Margulis et al. 1989).

Description of methods

Cultures

A list of the cultured taxa used in this study is presented in Table 1; the majority of these cultures were established as part of the EU CODENET project. Species of *Chrysochromulina*, *Imantonia* and *Isochrysis* were grown as batch cultures (0.5–2 L) in Erlenmeyer flasks with filtered, autoclaved seawater diluted to 30 PSU. Nutrients, vitamins and trace metals were added as in IMR 1/2 medium (Eppley et al. 1967) supplemented with 10 nM selenite. Other cultures were grown in f/2 (Guillard and Ryther 1962). Typically, cultures were grown at 17°C under white fluorescent light with a quantum flux of 50–100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and a 12:12 h light: dark cycle. Cultures were harvested by filtration or centrifugation.

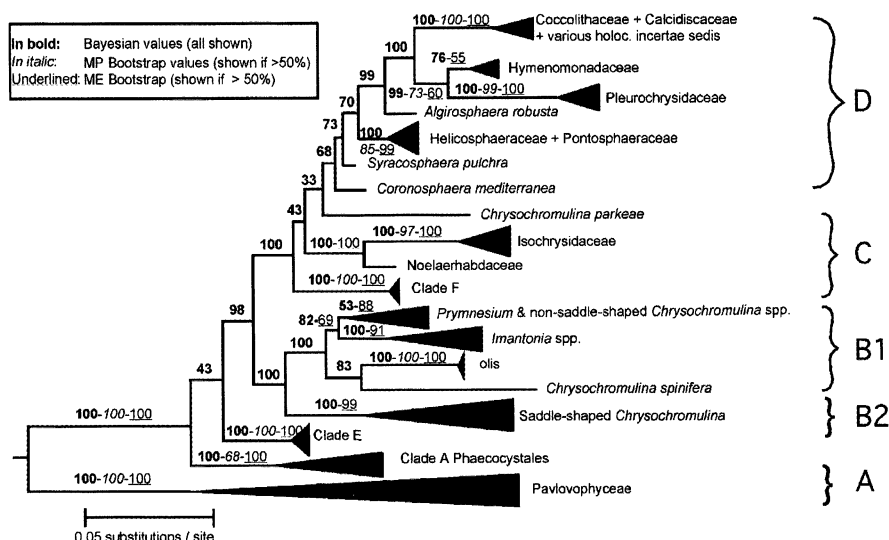


Fig. 1. Phylogenetic tree based upon a Bayesian analysis showing the relationships of haptophyte taxa. The tree is rooted on the branch leading to *Fucus* and *Cryptocodinium*, which have been pruned from the tree. For discussion of Clades A–F, see text. Maximum parsimony (MP) and minimum evolution (ME) analyses produced relatively similar trees, especially for younger nodes (not shown). Bootstrap values (500 replications) are represented at internal nodes for values > 50% for MP and ME analyses, respectively.

Table 1. Taxonomic list of the investigated species

We have organized them in a higher taxonomical framework based in our interpretation of the tree shown in Fig. 1 and indicated where taxonomic changes are warranted. Cultures of most of these species can be found at CCMP (USA) <http://ccmp.bigelow.org> or at ALGOBANK (France) <http://www.unicaen.fr/unicaen/ufr/ibba/lbbm/algobank>.

Division Haptophyta Hibberd ex Edvardsen et Eikrem

Class Pavlovophyceae (Cavalier-Smith) Green et Medlin

Order Pavlovales Green

Family Pavlovaceae Green

Species: *Diacronema vlkianum*, *Exanthemachrysis gayraliae*, *Rebecca salina*, *Pavlova gyrans*, *P. virescens*, *P. sp.* ('*pseudogranifera*'), *P. lutheri*, *Pavlova sp.* CCMP 1416, *Pavlova sp.* CCMP1394, *Pavlova sp.*

Class Prymnesiophyceae Hibberd

Order Phaeocystales Medlin **CLADE A**

Family Phaeocystaceae Lagerheim

Table 1. (cont.)

Genus: *Phaeocystis jahnii*, *P. cordata*, *P. globosa*, *P. antarctica*, *P. pouchetii*, *Phaeocystis* sp. PLY 559

New Order CLADE E

OLI16010, OLI51080, OLI26047, OLI51076

Order Prymnesiales (Papenfuss) Edvardsen et Eikrem **CLADE B**

Family Prymnesiaceae Conrad ex. O. C. Schmidt, **CLADE B1**

Species: *Imantonia rotunda*, *Imantonia* sp., *Imantonia* sp. CCMP1404, *Chrysochromulina* sp. CCMP 1204, *Prymnesium calathiferum*, *P. annuliferum*, *P. faveolatum*, *Prymnesium* sp. ('mediteranneum'), *P. nemamethecum*, *P. parvum* f. *parvum*, *P. parvum* f. *patelliferum*, *P. zebrium*, *Prymnesium* sp. ('tunis'), *Prymnesium* sp., *Platychrysis pigra*, *P. simplex*, *P. pienaaraii*, *Chrysochromulina polylepis*, *C. aff. polylepis* PLY 200, *C. chiton*, *C. kappa*, *C. minor*, *C. aff. herdlensis*, *C. hirta*, *C. ericina*, *C. fragaria*, *C. brevifilum*

New genus OLI33056, OLI51059

New genus *Chrysochromulina spinifera*

New Family CLADE B2

Species: *Chrysochromulina* cf. *ephippium*, *C. scutellum*, *C. strobilus*, *C. campanulifera*, *C. cymbium*, *C. simplex*, OLI16029, OLI51102, OLI26017, OLI 16108, *C. leadbeateri*, *C. parva*, *C. acantha*, *C. throndseni*, *C. rotalis*, *Chrysochromulina* sp. 1

New Order CLADE F

OLI26041, OLI51050

Order Isochrysidales (Pascher) Edvardsen et Eikrem **CLADE C**

Family Isochrysidaceae (Bourrelly) Edvardsen et Eikrem

Species: *Isochrysis galbana*, *I. litoralis*, *Isochrysidaceae* sp., cf. *Isochrysis* sp. 1, *Chrysotila lamellosa*

Family Noëlaerhabdaceae Jerkovic

Species: *Emiliania huxleyi*, *Gephyrocapsa oceanica*

Order Coccolithales (E. Schwarz) Edvardsen et Eikrem **CLADE D**

New Family (suggest new family when its position will have become stable in the Coccolithales)

Genus: *Chrysochromulina parkeae*

Family Pleurochrysidaceae Fresnel et Billard

Species: *Pleurochrysis scherffelii*, *P. carterae*, *P. carterae* v. *dentata*, *Pleurochrysis* sp., *P. placolithoides*, *P. elongata*, *P. roscoffensis*, *P. pseudoroscoffensis*, *P. gayraliae*, *Pleurochrysis* sp. 1, *Pleurochrysis* sp. 2 CCMP 875, *Pleurochrysis* sp. 3 CCMP 300

Family Coccolithaceae Poche

Species: *Coccolithus pelagicus*, *Cruciplacolithus neohelis*

Family Calcidiscaee Young et Bown

Table 1. (cont.)

Species: *Oolithotus fragilis*, *Calcidiscus leptoporus*, *C. quadriperforatus*, *Umbilicosphaera sibogae*, *U. foliosa*, *U. hulburtiana*

Family Reticulosphaeraceae Cavalier-Smith

Genus: *Reticulosphaera japonensis**

NB *Reticulosphaera* Grell is a non-calcifying protist very different from any known haptophyte, DNA sequences obtained by Cavalier-Smith et al. 1996 from cultures of *Reticulosphaera* are unquestionably of haptophyte origin but we suspect a haptophyte contaminant may have been present in the culture.

Family Hymenomonadaceae Senn

Species: *Jomonolithus littoralis*, *Ochrosphaera neapolitana*, *O. verrucosa*, *Ochrosphaera* sp., *Hymenomonas globosa*, *H. coronata*

Family Rhabdosphaeraceae Haeckel

Genus: *Algirosphaera robusta*

Family Helicosphaeraceae Black

Species: *Helicosphaera carteri* var. *carteri*

Family Pontosphaeraceae Lemmermann

Species: *Scyphosphaera apsteinii*

Family Syracosphaeraceae Lemmerman

Species: *Syracosphaera pulchra*, *Coronosphaera mediterranea*

Holococcoliths incertae sedis: *Calyptrosphaera* sp. 1, *Calyptrosphaera* sp. 2, *Calyptrosphaera sphaeroidea*, *Holococcolithophore* sp. 1, *Helladosphaera* sp. 1

Here we follow Edvardsen et al. (2000) in classifying all coccolithophores in two orders, the Isochrysidales and Coccolithales, with a diverse set of families in the Coccolithales. This has the advantage of maintaining (rightly or wrongly) the 1–2% molecular divergence between the orders of the haptophytes as in other algal groups where a molecular classification has been presented. In fact, our sample set does not allow us to test robustly the monophyly of the recently proposed additional orders, and a final decision as to whether to subdivide the Coccolithales will depend in part on further molecular analysis if more species become available in culture.

DNA extraction and PCR-amplification

Mainly for non-coccolithophores

Total nucleic acids were extracted using a modified CTAB extraction (Doyle and Doyle 1987) and served as a template for amplification of the 18S rRNA gene following Medlin et al. (1988) or Chesnick et al. (1997). Edvardsen et al. (2000), and references therein, offer further description of the methods. The PCR products were directly sequenced using a solid phase sequencing method with radioisotopes (Chesnick et al. 1997) or cycle-sequenced (Sequi-therm, BIOZYM) using infrared-labeled primers and analyzed with the LiCor automated sequencer (MWG), whereas others were cloned (LigAator, R&D Systems) prior to automated sequencing or were gel-purified before solid phase sequencing (Potter et al. 1996).

Mainly for *coccolithophores*

For a more detailed account of our experimental methods see Van Lenning et al. (2003). Genomic DNA was extracted with Qiagen "Dneasy Plant Minikit". Four oligonucleotides were used as sequencing primers of each template: those that were also used in the amplification reactions, 1F: 5'-aacctggtgacctgccagt, and 1528R: 5'-tgatccttctgcaggttcacctac from Medlin et al. (1988), plus two internal ones, F743: 5'-tgggataatgaaataggac, and R783: 5'-ccctaacttcgttcttg. The amplified DNAs were cleaned with a Qiaquick PCR Purification kit (Qiagen). Sequences from the PCR templates (employing both DNA strands) were fully resolved using an ABI 377 sequencer (Applied Biosystems) and dye terminator cycle sequencing kits (Perkin-Elmer Co.; reactions run by SeqLab). The quality of electropherograms was checked when editing sequences of DNA, and revised for confirmation of newly observed substitutions. Xesee 3.2 software (Eric Cabot) was used to align the sequences manually during collection and edition, and electropherograms were viewed with the Chromas 1.45 program (Conor McCarty).

Phylogenetic analyses

Sequences were manually aligned in an algal database containing over 300 published and unpublished chlorophyll a+c algae using maximum primary and secondary structural similarity with the Olsen sequence editor (Larsen et al. 1993). This data set also includes 14 sequences from a clone library obtained from amplified 18S rRNA genes from water samples taken in oligotrophic Pacific waters (Moon-Van der Staay et al. 2000). A final data set of 127 sequences was used for phylogenetic analyses with the brown alga *Fucus* and the dinoflagellate *Cryptothecodinium* as outgroups pruned from the tree. A total of 1712 nucleotides were used for the data analysis of which 358 were considered informative for parsimony analysis. Prior to any phylogenetic analysis, the data set was analyzed by the Modeltest program (Posada and Crandall 1998; Sáez and Medlin 2002) to determine the model of evolution that best fit the data set. This program selected the General-Time-Reversible (Rodríguez et al. 1990) and the Tamura-Nei models (Tamura and Nei 1993), both with a gamma distribution and allowing for invariant positions. For each selected model, the parameters for base variation, gamma, and number of invariant positions were then loaded into PAUP* (Swofford 2000), which was used to run the distance (minimum evolution; or ME) analyses, where distances were calculated by maximum likelihood. The topology of the trees obtained using both models of nucleotide substitution was identical. Maximum parsimony (MP) analyses were implemented with PAUP* as well. This procedure was carried out by weighting substitution types. Weight assignments were performed with MacClade 3.04 software (Maddison and Maddison 1992) using default options. The substitution types were weighted inversely to their observed frequency on an unweighted maximum parsimony tree. In all our analyses introduced gaps were treated as missing data. Stability of monophyletic groups in weighted maximum parsimony and distance trees was estimated with a bootstrap analysis (500 repli-

cates; Felsenstein 1985). For ME, the bootstrap values shown in Fig. 1 are the averages of those obtained under each of the models of nucleotide substitution, which gave very similar results anyway. We also ran a Bayesian search for the trees with higher posterior probabilities using MrBayes 2.01 (Huelsenbeck and Ronquist 2001). We ran it for each model selected by Modeltest with undefined gamma distributions and proportion of invariable sites, during 2 million generations for each model, and saving every 100th tree. We discarded the first 2,000 trees of each run, making sure that at the 2,001st tree the posterior probability was not significantly different than in the 2,000,000th tree. In this way, we collected 18,000 trees of high posterior probability under each model. Additional runs of 1 million generations were done to check that our results were not dependent on the initial trees picked at each run. Then we made a consensus tree with the 36,000 collected trees (18,000 from each model). On this consensus tree (Fig. 1), “credibility values” for each clade are shown, which represent the percentage of times that the correspondent clade is found among those 36,000 trees.

Description of phylogenetic clades

A phylogenetic reconstruction of the haptophyte algae based on nucleotide sequences of the 18S rRNA gene is presented in Fig. 1. We have collapsed the tree into higher taxonomic levels for ease of viewing the relationships among the families, orders, and classes. A list of all taxa in the tree and their assignment to higher taxa can be found above, in Table 1. The tree presented is the consensus tree from the Bayesian analysis, and bootstrap values from the MP and ME analysis, if higher than 50%, are also plotted onto the tree. When interpreting the reliability of a phylogenetic tree there cannot be certainties; however, if a node is well supported by a variety of different phylogenetic methods (such as the ones used here), the expectancy for the correspondent clade to be right can be very high. Bayesian methods (as the related but more computing intensive Maximum Likelihood) use more information from the data (DNA or protein sequences) than distance methods, and are less prone to artifactual errors than parsimony, and thus they are generally favored (Holder and Lewis 2003). That is why we have chosen a Bayesian tree to show (Fig. 1) and why we suggest relying somewhat more on that analysis.

All analyses recovered a major split in the haptophyte algae corresponding to its two classes: the Prymnesiophyceae and Pavlovophyceae. In the class Pavlovophyceae, Van Lenning et al. (2003) have shown that there are three major groups within the class, which can be defined by morphological and pigment features. *Exanthemachrysis* is the basal divergence followed by *Rebecca salina*. Then there is a split in the group with true *Pavlova* spp. forming one clade (this clade is defined as ‘true’ *Pavlova* because it contains the type species *P. gyrans*), sister to a clade containing *Diacronema* and *Pavlova* species with different stigmata and pyrenoids from those in the true *Pavlova* spp. clade. For example, in *P. lutheri* and *Diacronema vlkianum*, the stigma is composed of a layer of osmiophilic (lipid-

like) droplets close to or beneath the cell membrane. In *D. vlkianum*, it is associated with a groove in the surface of the cell and a specialized swelling on the shorter flagellum (Green and Hibberd 1977; Green 1980).

In contrast to the Pavlovophyceae, motile cells of the Prymnesiophyceae usually have their flagella apically inserted. The two flagella are smooth and the haptonema may vary from a few micrometers in species of *Phaeocystis* and *Prymnesium* to more than 100 μm in some species of *Chrysochromulina* or, may be lacking entirely (*Imantonia rotunda* and *Emiliania huxleyi*). The morphological and ultrastructural features of taxonomic importance for the prymnesiophycean species include cell shape, features of the haptonema (e.g., length, ability to coil, number of microtubules in the emergent part, presence of scales), scale investment, scale form, calcification, presence or absence of under-layer scales, presence or absence of resistant base plates, and presence of a continuous outer investment or 'skin', pyrenoid type, flagellar apparatus (e.g., presence or absence of compound roots and a cytoplasmic tongue, the number of microtubules in the sheet of the R1 root of the mature flagellum, and the presence or absence of helical structures in the flagella) (Edvardsen et al. 2000).

In the class Prymnesiophyceae, several major clades can be recognized (Fig. 1). Selecting taxonomically significant clades is obviously not an objective exercise. A reasonable approach seems to look for consensus with previously recognized taxonomical units. The clades that we propose at the order level in the taxonomy of the Haptophyta are: (1) Clade A = Phaeocystales; (2) Clade B = Prymnesiales; (3) Clade C = Isochrysidales; and (4) Clade D = Coccolithales. All these clades have a strong Bayesian support, being clade D the less well supported (68%). In addition, a series of sequences from a gene clone library obtained from oligotrophic Pacific waters are present (Clade E and Clade F, see Moon-Van der Staay et al. 2000). The morphology of members of these clades is unknown but oligonucleotide probes have been made to these clades in the hope of retrieving the cells in a FISH (fluorescent *in situ* hybridization) format. We leave *Chrysochromulina parkeae* out from any of these clades because its position within the "macroclade" made by F+C+D is uncertain, although there is a 100% Bayesian support for its belonging to this group (Fig. 1). Clade E represents a group of taxa with some affiliation to *Phaeocystis* spp.; it is an instable clade in the tree with two alternative positions, either the one shown in Fig. 1, or as a sister group to the Phaeocystales. Clade F is at the base of the Coccolithales and Isochrysidales. Because both clades, E and F, are clone library sequences we cannot comment on anything related to their phenotypes other than their size. All of the OLI clones are from the picoplankton fraction of natural samples, so these cells must be smaller than 3 μm . Either these are very small haptophytes or we have picked up gametes or zoospores in the water sample. Clearly these clades represent novel haptophyte taxa.

CLADE A – Phaeocystales

The order Phaeocystales contains, according to our molecular data, at least six distinct species. *Phaeocystis scrobiculata* was not included in our molecular study,

but, on morphological grounds, is believed to represent a seventh species. On the basis of the molecular data at least three colonial species are recovered (Medlin et al. 1994). These include *P. globosa*, *P. antarctica*, and *P. pouchetii*. Gene clone OLI51004 is included in this clade and is most closely related to *P. globosa*, but is not identical (Moon-Van der Staay et al. 2000). The unicellular warm water *Phaeocystis jahnii*, *P. cordata*, and an undescribed *Phaeocystis* sp. PML 559 are basal in the Phaeocystales and give rise to the colonial species. *Phaeocystis* likely arose as a warm water unicellular genus, which later diversified into colonial species that spread into both polar regions (Lange et al. 2002).

CLADE B – Prymnesiales

Usually, the non-motile and flagellate cells of the Prymnesiales are scale- and/or coccolith-covered. The ornamentation of both structures may vary from simple to very elaborate.

The genera belonging to the Prymnesiales (Clade B) can be divided into two sub-groups, Clade B1 and Clade B2 according to the 18S rRNA tree (Fig. 1; also the same in Edvardsen et al. 2000), and they correspond to Clade 1 and Clade 2 in the analysis of *Chrysochromulina* spp. by Simon et al. (1997). Both groups are well supported in the Bayesian analyses. Species of *Chrysochromulina* fall into both clades, and thus this genus must be considered paraphyletic (Inouye 1997; Simon et al. 1997; Fujiwara et al. 2001). It has previously been recognized on morphological grounds that *Chrysochromulina* is not a natural group (Birkhead and Pienaar 1995) and a revision of the genus is underway (Eikrem et al. unpubl.). Clade B1 contains *Imantonia* spp. as sister to a clade including certain non-saddle shaped *Chrysochromulina* spp. and all *Prymnesium* spp. This clade should correspond to the Family Prymnesiaceae. Within a sub-clade B1, the position of the clone library taxa (OLI51059 and OLI51033 + OLI51056) is sister to *Chrysochromulina spinifera*, another flagellate with a distinct morphology (Fournier 1971). Clade B2 contains only *Chrysochromulina* spp. that are saddle shaped, including the type of the genus, *Chrysochromulina parva*. This clade should be given a new family name, but retain the generic name *Chrysochromulina*.

CLADES C, D, & F

There is strong support in Bayesian analysis (100%; Fig. 1) for the clade containing all taxa bearing calcified scales (orders Isochrysidales and Coccolithales, clades C and D, respectively, together with clade F), providing a reasonable basis for the hypothesis that calcification has only arisen once in the evolution of the Haptophyta. This observation was also inferred from biomineralisation characteristics (Young et al. 1999), and was suggested previously, also from a molecular perspective, by Edvardsen et al. (2000) and Fujiwara et al. (2001). However, in relation to the taxa belonging to the "macroclade" comprising clades C, D and F,

is unknown if the members of this last clade F calcify or not, because of the fact that the corresponding DNA sequences were isolated from a clone library. *Chrysochromulina parkeae* clearly falls in the C+D+F group, and slightly calcifies (R. Andersen, pers. comm.). At present there is no taxonomic rank that unites clades C and D, which would likely be at the supra-order level.

CLADE C – Isochrysidales

This clade includes members of the families Isochrysidaceae (*Isochrysis*) and Noëlaerhabdaceae (*Emiliania* and *Gephyrocapsa*). The relationship of *Isochrysis* to *Emiliania* and *Gephyrocapsa* has been noted previously, and on the basis of possession of a vestigial haptonema, these three genera, plus *Imantonia*, *Dicrateria*, *Pseudoisochrysis* and *Chrysotila* were included in the Isochrysidales by Parke and Dixon (1976, see also comments by Green and Pienaar 1977). The validity of this character was questioned by Green and Jordan (1994) and molecular data has indeed shown that *Imantonia* is a member of the Prymnesiales (Edwardsen et al. 2000). However, the molecular evidence of Edwardsen et al. (2000) strongly supported separation of the other genera so they reinstated the order Isochrysidales. Young and Bown (1997b) independently argued, on grounds of coccolith ultrastructure, that the Noëlaerhabdaceae should be placed in a separate order, also including the fossil family Prinsiaceae. Their classification was exclusively based on coccolith morphology they introduced a new order, the Prinsiales, for these families. Because the molecular data has supported the reinstatement the order Isochrysidales, this name has priority over Prinsiales. The four genera of the Isochrysidales produce long-chain saturated alkenones and these have not been identified in any other haptophytes (see references in Jordan and Chamberlain 1997; Stoll and Ziveri this volume), so this appears to be a unique feature for the order. As noted above, calcification is likely to have evolved only once in the haptophytes, in which case it must be inferred that in the Isochrysidaceae calcification has been lost secondarily (Edwardsen et al. 2000; Fujiwara et al. 2001).

CLADE D – Coccolithales

The clade including the Coccolithales is less well supported (68%). It contains a diverse set of coccolithophores all of which have well-developed base-plate scales. We have chosen not to include the non-calcifying flagellate *Chrysochromulina parkeae* within this clade (or within any other) because of its uncertain position within the group composed of clades F+C+D (Fig. 1). *C. parkeae* falls at the base of clade D with a Bayesian support of only 33%; however, its belonging to the macroclade F+C+D is supported by a 100% Bayesian credibility value. Thus, this species may be closely related to the ancestral state from which all coccolithophores evolved, or at least those of clade D. In fact, *Chrysochromulina parkeae* has an ultrastructure like other coccolithophores and slightly calcifies (R. Andersen, pers. comm.).

At the apex of the clade is a very well supported sub-clade consisting of the Hymenomonadaceae, Pleurochrysidaceae, Coccolithaceae, Calcidiscaceae and

several poorly characterized holococcoliths. The other members of clade D are divided into several sub-clades, which correspond more or less to groups at the family level. The Hymenomonadaceae and Pleurochrysidaceae form discrete sister clades, which agrees well with predictions from cytology and ecology; they are both coastal groups, with non-calcifying haploid phases in the life cycle. Coccolith structure is somewhat different: the Hymenomonadaceae have rather simple coccolith structures relative to the Pleurochrysidaceae. A more in-depth discussion of the relationships of the taxa within this clade will appear in a subsequent publication (Sáez et al. unpubl.).

The other members of clade D are all coccolithophores for which there was no previous molecular data and which were of very uncertain relationships. They belong to five genera and four families, and in the classification of Young and Bown (1997b), to three orders. In general, the very low sampling makes it difficult to draw strong conclusions and we note that many bootstrap values are rather low and that different types of phylogenetic analyses (not shown) produced different relationships between these taxa. A revision of the classification would therefore be premature, nonetheless several valuable observations can be made.

1. Clearly, *Coronosphaera*, *Syracosphaera*, *Helicosphaera*, *Scyphosphaera* and *Algirosphaera* occupy an intermediate position between the Isochrysidales and Coccolithales (*sensu* Young and Bown 1997b). This was a robust result from all of our analyses. In some analyses they formed a separate clade, in others, as here, a paraphyletic grouping in the stem of the clade including the Coccolithales. A likely interpretation of the variable results is that the divergence between these groups dates back to the Early Jurassic radiation of the coccolithophores described by Bown (1987).
2. *Helicosphaera carteri* and *Scyphosphaera apsteinii* are sister taxa in all analyses. This is an interesting result; their coccoliths have very different shapes and they are classified in separate families, the Helicosphaeraceae and Pontosphaeraceae. However, the coccoliths do share various structural characteristics and on stratophenetic grounds these two families have both been inferred to have evolved from the extinct family Zygodiscaceae in the Paleogene (Romein 1977; Aubry 1989). Young and Bown (1997b) placed these families in the order Zygodiscales.
3. The two species of the Syracosphaeraceae, *Syracosphaera pulchra* and *Coronosphaera mediterranea* cluster together, although in different clades. This is a species-rich family so further analyses are likely to resolve this, however, it is worth noting that *C. mediterranea* is an atypical member of the Syracosphaeraceae in terms of coccolith structure so it would not be entirely surprising if it proved to be phylogenetically distinct from the true Syracosphaeraceae.
4. *Algirosphaera robusta*, the only representative of the Rhabdosphaeraceae cultured to date (Probert and Houdan this volume) appears to be closely related to the crown group of clade D. However, in terms of coccolith structure and cytology it shows more affinities to the Syracosphaeraceae (Probert et al. unpubl.).

In a recent study, Sáez et al. (2003) published a tree for the Coccolithaceae and Calcidiscaceae families (Table 1). Although discussed in a different context, i.e. genotypic variation among isolates from the same species or genera, their results, based in DNA sequences for the more variable plastid *tufA* gene, showed a tree for the mentioned families. Further work will be done to assess the phylogenetic value of this or alternatives trees based on *tufA* (Sáez et al. unpubl.), but, based on the performed analyses, we have good support for the referred tree, also reproduced in Fig. 2 of de Vargas et al. this volume. Those results reveal a monophyletic origin for each of the genera *Umbilicosphaera* and *Calcidiscus*, as well as for the *Oolithotus* + *Calcidiscus* combination. In turn, all these taxa form one clade with *Coccolithus pelagicus* as the sister taxon. Finally, *Cruciplacolithus neohelis* branches out at its base. This establishes a close relatedness for the members of all discussed genera, and for *Oolithotus* in relation to *Calcidiscus*, which is not surprising. Even more interesting, it showed a close relationship, but with deep divergence, between *Cruciplacolithus* and *Coccolithus*.

CLADE F

This clade originates from the clone library samples, i.e. DNA sequences isolated from bulk genomic DNA purified from filtered water samples, and thus, has an unknown morphology.

Conclusions

It is encouraging to note that in general the molecular data available for the Haptophyta supports the systematic schemes based on traditional morphological information. The uniqueness of some of the clades from the gene clone library (Clades E & F) in terms of their molecular relatedness to other known cultured haptophyte species suggests that there may be many novel as yet undescribed or unseen haptophyte taxa, even coccolithophores in the world's open oceans.

A summary of the taxonomic ranks above the genus level supported by molecular, morphological and ultrastructural analysis is listed in Table 1. More complete checklists of genera in each family can be found in Jordan and Green (1994) and Young and Bown (1997b). Their inclusion in each family is based on morphological and ultrastructural evidence. The detailed, revised and formal descriptions of the orders and families, other than the Coccolithales, confirmed by molecular analysis can be found in Edvardsen et al. (2000) and in a future publication by Eikrem et al. (unpubl.). For coccolithophores, a formal original publication with the new reported DNA sequences is also under preparation (Sáez et al. unpubl.).

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***Super-Species* in the calcareous plankton**

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Summary

The most successful groups of pelagic protists in the modern Ocean have evolved convergent phenotypic traits, including the presence of hard skeletons enclosing the cell. These micro-skeletons – tests, coccoliths, frustules, theca – have accumulated kilometers of deep-sea sediments since the Jurassic, the most complete and continuous fossil record widely used for reconstructing Earth systems dynamics and microbial evolution. The use of the traditional morphological species concepts in those groups indicates that the relatively few species living at a given time have huge, often circum-global biogeographic distributions, and commonly last for many million years in the sediment record, which contrasts with the hectic biological pace of life occurring in the oceanic water masses, leading to one of the highest organismic turnover that any ecosystem sustains. Here we review all recent genetic data on coccolithophore and foraminifer biodiversity. In both groups, the sequencing of various genes shows that the *morphological ‘species’* are in fact monophyletic assemblages of *sibling species* which diverged several million years ago according to molecular clock calculations. Furthermore the sibling species within a morphological entity may systematically occupy restricted geographic or temporal *allopatric* subdivisions of the total ecological range attributed to the traditional morphospecies. They display also stable and subtle – despite million years of genetic isolation – morphological differences that have been previously overlooked or interpreted as ecophenotypic variations. Obviously, various selective forces related to life in the marine planktic realm impose a strong stabilizing selection on pelagic organisms that maintains “optimal” phenotypes through the origination and possibly extinction of sibling species. We propose that this mode of evolution is characteristic of most marine planktic taxa, including metazoans, and we introduce a concept of ‘*planktic super-species*’ to describe these constrained morphological monophyletic entities that include several sibling species

adapted to different ecological niches. Two different evolutionary models displaying different degrees of complexity in the spatio-temporal disconnection between morphological and genetic/ecologic differentiations are discussed in the frame of the existing morphometric and DNA data sets. The design of experimental protocols at the boundary between molecular phylogenetics and micropaleontology will be a necessary condition to test which of our models reflect the real world. This will be also a crucial step to reveal the full potential of microfossil applications in paleoecology and stratigraphy, and to understand, at the level at which adaptation and selection operate, how pelagic biodiversity reacted to climatic changes in the past oceans and how it may react to the severe warming events projected in the near future.

Open questions in the open ocean

Open ocean biosphere

The evolution of Earth ecosystem and global climate is largely dependent on the origination, maintenance, and extinction of *biological species*¹ that ultimately regulate the distribution and cycling of certain elements. Although the open ocean is recognized as one of the most important and active compartments for biogeochemical cycles, the *diversity, biogeography and evolutionary processes* characterizing the pelagic biosphere are still largely unknown at the level of the species.

The relatively poor knowledge of open ocean biota is mainly due to the immense dimensions of the pelagic environment, the small and highly diluted biomass, and the historical delay in the study of the marine plankton, which started only in 1872/76 with the *Challenger* expedition. Indeed, whereas the pelagic realm is *gigantic*, most planktic taxa are *minute* and display a relatively restricted range of shapes by comparison to terrestrial or coastal benthic organisms. The planktic biodiversity is largely dominated by micrometric cells (protists and prokaryotes) and viruses, whose species-level taxonomy, geographic distribution, and ecology are far from being understood.

Another obstacle to our understanding of the pelagic ecosystem is the unique pace of both water masses and populations dynamics, which challenges biological sampling. Most microscopic pelagic organisms are characterized by phases of exponential growth (sometimes even blooms) when the physical, chemical, and biological conditions are favorable, followed by phases of population destruction

¹ We do not want to enter into the “species debate” here (Hey 2001). The idiom “*biological species*” contrasts with the “*morphological species*” or “*morphospecies*” discussed later in the text. We define a *biological species* as a group of related organisms sharing evolutionary processes. Genes are exchanged at varying rates between the individuals within the evolutionary group, which maintains a certain genomic and thus functional cohesion within the *biological species*.

or dormancy (Steinberg et al. 2001). The life spans of the pelagic unicellular organisms range from a couple of hours to a day for most picoplanktic species (Vaulot et al. 1995), to a few days or weeks, rarely more than a season, for the nano- and microplankton. This intense rate of organismic turnover may be an adaptation of the pelagic species to survive in a perpetually moving, three-dimensional environment. Individual cells drift in ocean currents at speeds of a few meters to kilometers per hour, and yet must be able to release enough offspring – and thus potential founders of new populations – before being transported into hostile conditions where they may not survive.

Those unique and extremely disparate spatio-temporal scales characterizing pelagic processes have for a long time discouraged biologists from studying the pelagial by analyzing its primary components, namely the biological species. Avoiding those difficulties and by exploiting progress in remote sensing, researchers in biological oceanography rather started to measure chemical or physical bio-markers as proxies for estimating global biological processes (e.g. Antoine et al. 1996; Li 2002).

Excellence and caveats of pelagic fossil records

However, comparable avoidance strategies have not been available to scientists approaching the pelagic ecosystem through the analysis of deep-sea sediments. There, the hectic pace of pelagic life is abruptly frozen; the continuous rain of skeleton-bearing microorganisms from the water column to the bottom of the ocean results in a compression of the temporal scale and immobilization of the spatial scale, which allows a global survey of pelagic diversity and biogeography only by analyses of individual skeletons. This fantastic potential of the pelagic fossil record and its use as a stratigraphic tool for oil industry and paleoclimatic reconstructions, promoted extremely detailed taxonomic analyses within the groups of skeleton-bearing pelagic cells (coccolithophores, diatoms, foraminifers, radiolarians). Based on morphological criteria, micropaleontologists have patiently described and classified into *morphological species*, the variability of the countless shells extracted from the worldwide deep-sea sediment archives (e.g. Bolli et al. 1985, Haq and Boersma 1998; CLIMAP 1981). The efficiency of this morpho-taxonomic system for stratigraphic purpose has been amply demonstrated (Bolli et al. 1985; Berggren et al. 1995). However, its relevance and accuracy for evolutionary and paleoclimatic studies are less clear, and may be limited by the scarcity of biological/ecological calibration studies of the morphological species concepts, often rashly utilized as a postulate in the numerous analyses assuming that a particular morphospecies represents a biological species with a specific habitat.

As a matter of fact, both biologists and paleontologists working on open ocean organisms have had a hard time with the use of a biological species concept for their ecological and evolutionary studies. It appears, however, fundamental to identify which *genetic entities* are responsible for the global “biological field” measured today from space, or for the phenotypic variability expressed in the

shells at the bottom of the ocean. How many genetic species are there for the well-known morphospecies? Where do they live and why? When did they appear, and which micro-evolutionary processes characterize their origin, maintenance, and extinction? The analyses of DNA sequences of *individual* pelagic cells, which have recently become possible, allow one to approach those questions and provide biological and evolutionary meaning closer to the level at which natural selection acts, to both water column and deep-sea sediment data. Here we will illustrate how the analysis of DNA sequences within the most important groups of calcareous unicellular plankton (Fig. 1) is beginning to change and will further modify our understanding of morphological and genetic variation at the species level in open ocean protists. The CODENET project produced the first molecular data challenging the morphological species concepts within the coccolithophores. Our aim in this chapter is not to enter into the details of the data (Sáez et al.

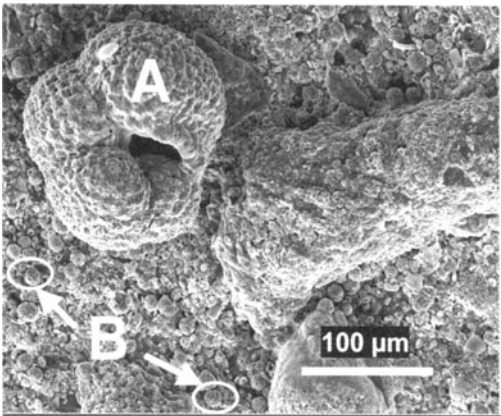


Fig. 1. Planktic foraminifers (A a juvenile specimen of *Neogloboquadrina pachyderma*,) and coccolithophores (B *Coccolithus pelagicus*) constitute the major component of deep-sea calcareous sediments that cover 66 % of the bottom of the global ocean (Milliman 1993). Fundamental biological/structural differences between both groups are reported below.

	Foraminifers (A)	Coccolithophores (B)
CaCO ₃ skeleton	A single calcareous test per cell growing by successive chambers addition. No naked cells detected yet.	10 to 100 minute calcareous platelets (liths) fixed at the surface of the cell. Frequent naked cells.
Adult size	50 to 1000 μm	3 to 25 μm
Nutrition ¹	Heterotrophs	Autotrophs
Life span	Typically half a month or a month, sometimes linked to lunar cycles (Hemleben et al. 1989)	Days to weeks
Life cycle	Probably exclusively sexual (2N)	Haplo-diplontic, with dimorphism associated to life cycle stages: holococcoliths (1N), heterococcoliths (2N)

¹ This distinction in the nutritional mode is not clear-cut as some coccolithophorid species may be mixotrophic or heterotrophic (Billard and Inouye this volume), and several foraminifers display obligatory symbiosis with phototrophic algae (Hemleben et al. 1989).

2003 and this volume; Geisen et al. this volume) but to synthesize and compare them with the molecular results obtained during the last six years within the planktic foraminifers (de Vargas et al. 1997–2002; Darling et al. 1996–2000; Stewart et al. 2001). The implications of both foraminifer and coccolithophore DNA data will be discussed with respect to pelagic diversity and biogeography, evolutionary phylogeny, and analyses of the fossil record.

Behind the phenotype

Since the beginning of methodical use of DNA mutations as a taxonomic tool and time machine, discoveries of new *cryptic*² or *pseudo-cryptic species* have been rising in every studied group of organisms, and mainly in those displaying simple morphologies (unicellular taxa, primitive or parasitic metazoans). Pseudo-cryptic speciation is a very commonly observed phenomenon among coastal marine organisms (Knowlton 1993, 2000). In the pelagic realm, it may even be the rule rather than the exception (de Vargas et al. submitted). Most common planktic *morphospecies*, often considered as cosmopolitan, that have been analyzed so far using DNA sequencing, were found to be composed of a complex of different genetic entities. They range from the pico- to the macro-fractions and include cyanobacteria and prochlorophytes (Fuhrman and Campbell 1998), haptophytes and diatoms (Medlin et al. 2001), dinoflagellates (Scholin et al. 1995), copepods (Bucklin et al. 1996), and fishes (Miya and Nishida 1997). As discussed below, this prevalence of genetic diversification with no or only subdued morphological differentiation may be due to the unique environmental and biological pressures on morphology endured by open ocean plankton.

DNA versus morphological diversity in calcareous unicellular plankton

Foraminifera

Among skeleton-bearing pelagic microorganisms, planktic foraminifers constitute an exceptional case, as they are among the biggest unicellular organisms whose shells can be rapidly manipulated with simple hand tools under a dissecting microscope. Their fossil record is often presented as one of the rare examples known at the “species” level (Bolli et al. 1985). Their presence and absence is

² Species considered morphologically identical, but discernible using genetic, physiological, or behavioral characters. In fact, the majority – if not all – of the new, genetically identified species within traditional morphological species, turn out to show minor but recognizable morphological characteristics after closer examination of the specimens. The term “pseudo-cryptic” species is applied in these cases.

Table 1: Current DNA data sets on the genetic variation at the morphospecies level in planktic foraminifers and coccolithophores. Species are ranked according to the amount and geographic extension of genetic data available. Abbreviations: SSU = Small SubUnit; ITS = Internal Transcribed Spacer; rDNA = nuclear ribosomal DNA; TufA = chloroplast transcription elongation factor; RFLP = Restriction Fragment Length Polymorphism data. Ref: 1. de Vargas et al. 1999; 2. de Vargas et al. in prep.; 3. Darling et al. 2000; 4. Darling et al. 2001; 5. Darling et al. 2003; 6. de Vargas et al. 2001; 7. de Vargas et al. submitted; 8. de Vargas, unpublished; 9. de Vargas et al. 2002; 10. Kucera and Darling 2001; 11. Stewart 2000; 12. de Vargas et al. 1997; 13. Darling et al. 1997; 14. Saez et al. 2003.

Morphospecies	Gene(s)	# of ge- netic types	# of specimens genotyped	# of stations, geographic locations	Ref.
Foraminifer					
<i>Orbulina universa</i>	SSU/ITS rDNA	3	764 (65 seq/700 RFLP)	102; large transects: Atlantic, Indian Ocean, South Pacific. See Fig. 3	1; 2
<i>Neogloboquadrina pachyderma (sin)</i>	SSU rDNA	4	263	~30; 4 transects in the North and South Atlantic	3; 4
<i>N. pachyderma (dext)</i>	SSU rDNA	2	66	~30; 4 transects in the North and South Atlantic, California	3; 4; 5
<i>Globorotalia truncatulinoidea</i>	SSU/ITS rDNA	4	350 4 SSUseq/50 ITS seq/230 RFLP	102; large transects: Atlantic, Indian Ocean, South Pacific. See Fig. 3	6; 7; 8
<i>Globigerinella siphonifera</i>	SSU rDNA	4	189 (25 seq/164 RFLP)	55; large transect in the Atlantic	9
<i>Globigerina bulloides</i>	SSU rDNA	6	85	27; North and South Atlantic, California	10
<i>Turborotalita quinqueloba</i>	SSU rDNA	3	24	22; North and South Atlantic	3
<i>Neogloboquadrina dutertrei</i>	SSU rDNA	3	A few	Caribbean, Azores, Coral sea, California	11; 5
<i>Globigerinoides ruber</i>	SSU rDNA	4	A few	Caribbean, Canary, Coral sea, California	11; 12; 13
Coccolithophore					
<i>Calcidiscus leptoporus</i>	TufA / SSU rDNA	3	13	6; East coast of North and South Atlantic, Mediterranean	14
<i>Coccolithus pelagicus</i>	TufA / SSU rDNA	2	9	7; East coast of North and South Atlantic, Mediterranean	14
<i>Umbilicosphaera sibogae</i>	TufA / SSU rDNA	2	2	2; West coast of North Atlantic, Mediterranean	14
<i>Pleurochrysis carterae</i>	TufA / SSU rDNA	2	2	2; East coast of North Atlantic, California coast	14
<i>Helicosphaera carteri</i>	TufA	2	2	2; East coast of South Atlantic, Mediterranean	14

widely used for biostratigraphic dating and their relative abundance changes for paleotemperature reconstructions (e.g. CLIMAP 1981). The analysis of calcite precipitated in foraminiferal shells has also been used for a number of other important paleoproxies, such as primary productivity, temperature, and atmospheric CO₂ concentration (see recent review by Fisher and Wefer 1999).

For molecular phylogenetic studies, single living planktic foraminiferal cells can easily be sorted after collection with a plankton net, and individually isolated for DNA extraction PCR amplification (de Vargas et al. submitted). This is a great advantage over the coccolithophores (Fig. 1) which require clonal growth, a sometimes tricky procedure, before genetic analyses.

The modern diversity of planktic foraminifera has been divided into only about 45 species (Hemleben et al. 1989), based on the same morphological criteria as used in the study of their fossil record (i.e. test's architecture, ornaments and texture). During the past six years, the application of DNA sequencing methods in planktic foraminifera fundamentally altered our way of thinking about evolution and ecology in the group. In fact, the eight morphological species that have been genetically analyzed to date using numerous individuals from several ocean basins and water masses (Table 1), *all* contain between three to six genetic entities (de Vargas et al. 1999, Darling et al. 1999, Stewart et al. 2001, de Vargas et al. 2001, de Vargas et al. submitted). Phylogenetic and phylogeographic (see below) analyses of the DNA sequences coding for the Small SubUnit and Internal Transcribed Spacer of the nuclear ribosomal RNA (SSU and ITS rDNA) indicate that the genotypes within these morphospecies are in fact distinct *biological sister species*, which may have already evolved millions of years ago.

Of primary importance are the observed genetic distances between the genotypes as a result of the very slow rate of a few mutations per million years within the SSU rDNA (Fig. 2). The calibration of these genetic distances – or molecular trees – using morphospecies divergence dates from the outstanding foraminiferal fossil record suggest that most of the sister species diverged back in the late Miocene (Fig. 2). In addition, reproductive isolation is supported by the fact that individual foraminifers never contained more than one type of ribosomal gene. Genetic mixing would be expected if the sister genotypes, that occasionally co-exist in the same watermass (see below), were not fully isolated and still hybridized today. Finally, the individuals within a sister species have been found to be identical within the SSU rDNA, regardless of segregation by huge geographic distances (de Vargas et al. 1999, Darling et al. 1999), current belts (Darling et al. 2000) or tectonic barriers (de Vargas et al. 2002).

Despite the fact that traditional taxonomic descriptions were based on single holotypes, *all* classically defined morphospecies allowed for a considerable range of morphological variability, albeit often poorly defined. In most cases, such variability had been related to biogeography and hydrographic regime and was interpreted as phenotypy expressed as geographic changes in the proportions of clinal variants (Kennett 1976). Recent examples include shell size (Schmidt et al. in prep.), coiling direction (Kucera and Kennett 2002), test shape (Spencer-Cervato and Thierstein 1997), or porosity (de Vargas et al. 1999).

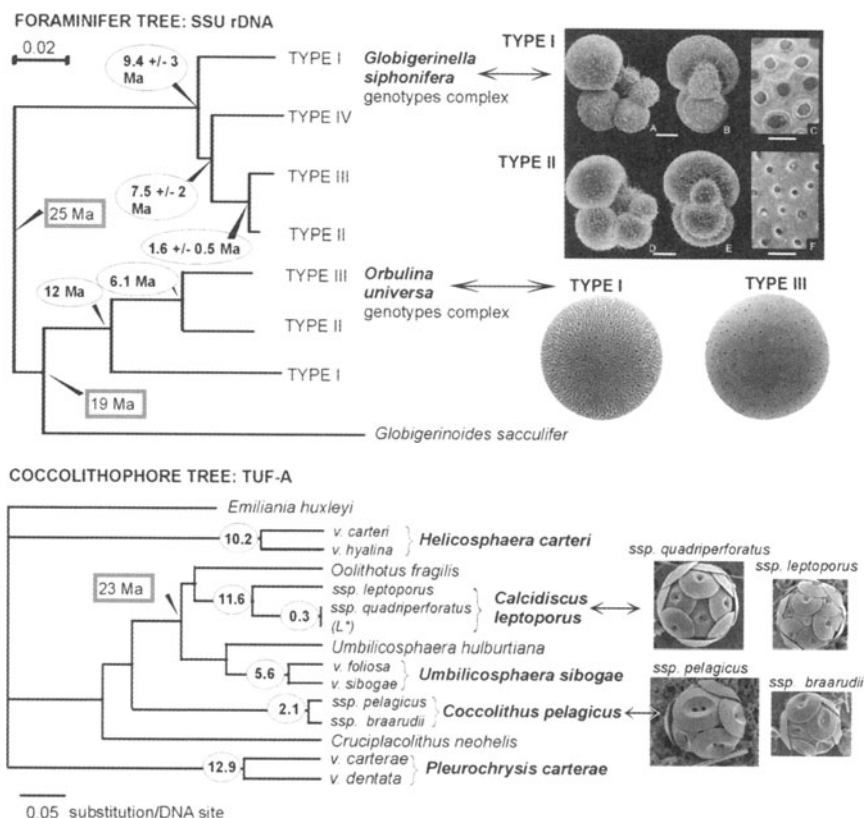


Fig. 2. Example of speciation within the classical morphospecies of foraminifers and coccolithophores. The phylogenetic molecular trees were calibrated with dates – in gray square – from the deep-sea fossil record of both groups, which allowed to estimate divergence dates between the sibling species within a morphological entity. Note that most sibling species evolved in the late Miocene, between 5–11 Ma. Details on the phylogenetic analyses are found in de Vargas et al. 1999, 2001 (foraminifer) and Saez et al. 2003 (coccolithophore). Four pairs of sibling species are shown to illustrate the striking morphological homogeneity despite millions of years of genetic isolation. (Pictures: *G. siphonifera*: Huber et al. 1997; *C. leptoporus* and *C. pelagicus*: CODENET images, see http://www.nhm.ac.uk/hosted_sites/ina/CODENET/GuideImages/).

The few recent quantitative studies that directly tested the relationship between morphologic and genetic differentiations in planktic foraminifera (*Globigerinella siphonifera* (Huber et al. 1997) and *Globorotalia truncatulinoides* (de Vargas et al. 2001) confirmed that subtle morphological variation(s) may characterize the newly identified genotypes. It is, however, still a major challenge to distinguish the morphological and/or geochemical variability of shells related to genetic isolation and evolution from that based on mere ecophenotypy or random

intraspecific polymorphism. The vastness of the three-dimensional ecospace characterizing the biogeographic range of unicellular plankton species constitutes the main barrier. Recently, de Vargas et al. (2001, submitted) developed a simple and efficient method to extract and preserve DNA from single living foraminiferal cells collected from the ocean while retaining their calcareous shell for further morphologic, chemical, or isotopic analyses. The method was applied during two basin-wide cruises in the Southern Indian and Southern Pacific Oceans (40 stations, Fig. 3). The authors were able, using PCR amplification and RFLP methods on a large number of individuals within the morphospecies *Globorotalia truncatulinoides*, to rapidly detect that smaller and more biconvex shells collected in the subtropical and polar frontal systems all around the world in the Southern Hemisphere, correspond to distinct genetic species.

Coccolithophores

Coccolithophores are about 100 times smaller than foraminifers. Their taxonomy is based principally on the morphological description of the micrometric calcareous *liths* or scales covering their cells and preserved in deep-sea sediments. However these coccoliths constitute only the single elements of the entire extra-cellular architecture of the organism – the coccosphere. As it is rare to find preserved intact coccospheres in sediments, isolated coccoliths or scales remain the only main taxonomic tool to describe extinct taxa. Nevertheless, the immense abundance of coccoliths in sediments and the fact that coccolithophores play a crucial role in global open ocean ecology and biogeochemical cycles (Westbroek et al. 1994) have fomented their study. In spite of the need to use optical combined with electron microscopes for morpho-taxonomic identification, their morphological diversity is the best known among any group of pelagic primary producers.

The modern diversity of coccolithophores has been divided into about 200 species (Jordan and Kleijne 1994, Billard and Inouye this volume) based on morphological criteria. This number of *morphospecies* is certainly overestimated, because evidence is increasing that most, if not all coccolithophores display complex haplo-diploid life cycles with the expression of two or even three radically different coccoliths during the different stages (hetero- and holo-coccoliths in the 2N and N stages, respectively) (Geisen et al. 2002). If this is confirmed, the ~70 holococcolith bearing “species” are in fact the phenotypic expression of the haploid life stage and their numbers will have to be subtracted from the total number of morphospecies resulting in a total extant species richness of ca. 130.

However, it is common to find consistent and subtle morphological differences within groups of heterococcoliths and their coccospheres ascribed to a single species (Jordan and Green 1994; Geisen et al. this volume; Quinn et al. this volume). The genetic dataset acquired during CODENET allowed assigning a biological meaning to some of these so-called “morphotypes”, “varieties”, or “formae” (Jordan et al. 1995). A molecular phylogenetic study (Sáez et al. 2003) within five morphospecies showed that *all* “species” were composed of two to three clearly distinct genetic entities (Table 1 and Fig. 2). The results, based on the chloroplastid gene *tufA*, were confirmed by additional analyses of the nuclear SSU and

ITS rDNA. As in the foraminifers, the total absence of DNA mutations between individuals within a genetic type, regardless of segregation by sometimes huge geographic distances, and the lack of hybrid genotypes despite the common co-occurrence of the varieties in the same water-column (Renaud and Klaas 2001), strongly supports full reproductive isolation of the genotypes (speciation). A molecular clock based on the fossil record (using, as a calibration date, 23 Ma for the stratigraphic origins of both *Umbilicosphaera* and *Calcidiscus*) allowed to estimate that most analyzed sister species separated prior to 2 Ma, most speciation events occurring, as for the foraminifers, back in the Late Miocene (Fig. 2).

Furthermore, the sister species within a morphospecies seem to be systematically discernible based on morphological characters, either structural as in the case of the “varieties” known from the fossil or living record (*sibogae/foliosa* of *U. sibogae*; *carteri/hyalina* of *H. carteri*; *carterae/dentata* of *P. carterae*, Fig. 2 and Geisen et al. this volume), or morphometric as the difference in coccosphere and lith sizes between the cryptic species within *C. leptoporus* and *C. pelagicus* (see Quinn et al. this volume; Geisen et al. this volume). In the CODENET data set, the small number of strains sequenced for each of these species, which come from a narrow portion of the full specific biogeographic range (samples were mainly collected from coastal waters of the Eastern Atlantic, Table 1) still limit the establishment of a link between morphologic and genetic differentiations. However, important lessons can already be learned, for instance from the most thoroughly studied case of *Calcidiscus leptoporus*. In this morphospecies, classical morphometric analyses in sediment samples from all around the world distinguished three morphotypes (large, intermediate, small) having coccospheres and coccoliths of different sizes (Knappertsbusch et al. 1997). The morpho-genetic analyses of 13 strains from coastal waters off Africa, Portugal, Ireland, and the Mediterranean, show that coccolith sizes overlap between the genetic types representing two of the classical varieties (intermediate and large) and size alone is therefore not an accurate tool of identification. On the other hand, a careful look at the morphology of the genetic species within *C. leptoporus* resulted in the discovery of a *discrete* morphological character that can be used to distinguish between the sister-species (the structure – *infilled* or *clear* – of the central area of the coccolith’s distal shield, see Quinn et al. this volume). This example illustrates how DNA analyses can help morphological taxonomy, which is particularly risky in coccolithophores where species concepts are based on analyses of single building blocks of the skeleton. Another aspect of the morphologic distinction between the sister species is discussed by Geisen et al. (this volume): the subtle, or even imperceptible (in *Syracosphaera pulchra* and *Coronosphaera mediterranea*) morphological differences among the heterococcoliths of the cryptic biological species maybe much more obvious in the holococcoliths produced during the haploid phase of their life cycle. The still very few cases of reported combination coccospheres between one heterococcolith type and two or even three holococcolith types seem in fact to result from low morphological differentiation (resulting in cryptic speciation) at the level of the heterococcoliths (Geisen et al. 2002). Here again, DNA sequences will be a powerful tool to rapidly establish links between life cycles and specific morphologies.

Water-mass and depth adaptation of genotypes

In the pelagial, most species of the phyto- and zooplankton have been reported as cosmopolitan, or at least as inhabiting huge, circum-global, and often bipolar – or antitropical – biogeographic ranges (Bé 1977, Roth 1994, Van der Spoel and Heyman 1983). The sea surface temperature gradient from the poles to the equator is often invoked as the most important limiting factor constraining the distribution of individual species, which in principle can rapidly be transported between basins or hemispheres through oceanic currents (Van der Spoel and Heyman 1983). This view of the biogeography of planktic microorganisms is mainly based on morphological species concepts, and has also been strongly influenced by the unique, global scale analyses of protistan shells retrieved from deep-sea sediments (Arnold and Parker 1999, Hemleben et al. 1989, Spencer-Cervato 1999).

The repeated discovery of sibling species displaying major genetic differences within all analyzed morphospecies of foraminifers and coccolithophores obviously challenges the widely held view of cosmopolitan plankton species distributions. It leads to new questions such as: do the sister species within a morphospecies inhabit the same oceanic province? Or do they show latitudinal, depth, or temporal allopatry? And which abiotic or biotic ecological factor(s) rule their distribution? Unfortunately these questions face a major sampling challenge. The biogeographic ranges of pelagic micro-biota are huge and three-dimensional. They have diffuse ecological boundaries and they are highly dynamic in space and through time. Besides, the large majority of open ocean planktic micro-organisms are very fragile and not viable in culture (Moreira and Lopez-Garcia 2002), often preventing the substitution of biogeographic analyses of wild populations with physiological laboratory experiments.

Planktic foraminifera

The recent application of simple, rapid and cost-effective protocols for single-cell PCR amplification (de Vargas et al. submitted) demonstrate that the methods are becoming sensitive enough to survey rapidly the taxonomy, phylogeny, and biogeography of pelagic protists at the individual level and over a global geographic range. The first large-scale study at the morphospecies level concerned the foraminifer *Orbulina universa* (de Vargas et al. 1999). It showed that the distribution of three cryptic species within *O. universa* along a 50°N to 50°S transect in the Atlantic is clearly not random, but strongly correlated with the degree of stratification – and thus productivity – of the water column. One sibling species was adapted to the vertically mixed, nutrient-rich and productive hydrographic regions (coastal upwelling, frontal zones in the subtropical and equatorial current systems), whereas the two others were collected in stratified sub-tropical water masses where the thermocline guards the surface layer from macronutrient input. We will call these ecological distributions “*transitional vertically mixed*” and “*sub-tropical stratified*”. In that early study, the coverage of the full latitudinal range of the morphospecies and of two opposite seasons in each hemisphere, suggested that the unveiled patterns and the partitioning of diversity might be ex-

tended to the global ocean. Since then, the genetic characterization of more than 700 individual cells isolated at 80 pelagic stations from the Atlantic, Indian, Pacific Oceans, and the Mediterranean and Red Seas (Fig. 3A) have confirmed the results of the Atlantic data set in an amazingly simple and predictable way (de Vargas, unpublished data). On the other hand, a basin-wide genetic analysis of another spinose morphospecies, *Globigerinella siphonifera*, which has a biogeographic range similar to that of *O. universa*, confirmed the importance of water mass stratification and nutrient input for the geographic segregation among the four identified sister species (de Vargas et al. 2002). The apparently cosmopolitan distribution of one of the genetic types of *G. siphonifera* may result from its adaptation to mesotrophic hydrographic conditions, the species living deeper at the top of the thermocline in stratified (sub)tropical waters. Analyses of shell morphology and chemistry ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$, Mg/Ca ratio) from this genetic type in the Caribbean (Bijma et al. 1998; Huber et al. 1997) also support our hypothesis of depth allopatry and emphasize the need to collect vertically stratified samples in the future.

At higher latitudes, the temperate to polar domains are mainly the realm of the non-spinose globorotaliid foraminifers (Hemleben et al. 1989). The best documented studies relate to the morphospecies *Globorotalia truncatulinoides* (de Vargas et al. 2001, submitted) and *Neoglobobulimina pachyderma* (Darling et al. 2000; Stewart et al. 2001; Darling et al. 2001) (Table 1). Both species have huge geographic ranges covering the subtropical to subpolar provinces (Hilbrecht 1996), with a typical asymmetric expansion toward the southern subpolar water-masses for *G. truncatulinoides* (Kennett 1968), and a unique bipolar extension into the polar domains for *N. pachyderma*. The latter is the only morphospecies of planktic foraminifer living at such high latitudes and it has been collected below or even in Antarctic ice. In deep-sea sediments from the southern hemisphere, *G. truncatulinoides* displays a typical morphological cline along the subtropical to subpolar ecological gradient (Kennett 1968, Healy-Williams et al. 1985). Application of single cell PCR amplification from more than 300 *G. truncatulinoides* individuals in the Atlantic (de Vargas et al. 2001), Indian (de Vargas et al. submitted), and Pacific (de Vargas, unpublished data) oceans, shows that this morphological cline is actually the expression of four different genetic types adapted to different latitudinal belts (Fig. 3B). Whereas two types were subtropical, the third one inhabited the highly productive subtropical frontal zone, and the fourth was collected far south in the cold subpolar frontal zone. For *G. truncatulinoides*, both fossil (Kennett 1970, Pharr and Williams 1987) and molecular clock data (de Vargas et al. 2001) independently suggest that those global eco-morpho-genetic evolutionary steps toward southern water-masses occurred at about 300–200 ka in this species complex, whose origins date back to 2.8 Ma (Lazarus et al. 1995). As for *O. universa*, the data set obtained from three basin-wide oceanic transects allowed coverage of most of the morphospecies' geographic range, and the distribution patterns found in different seasons, years, basins in the southern hemisphere were remarkably predictable.

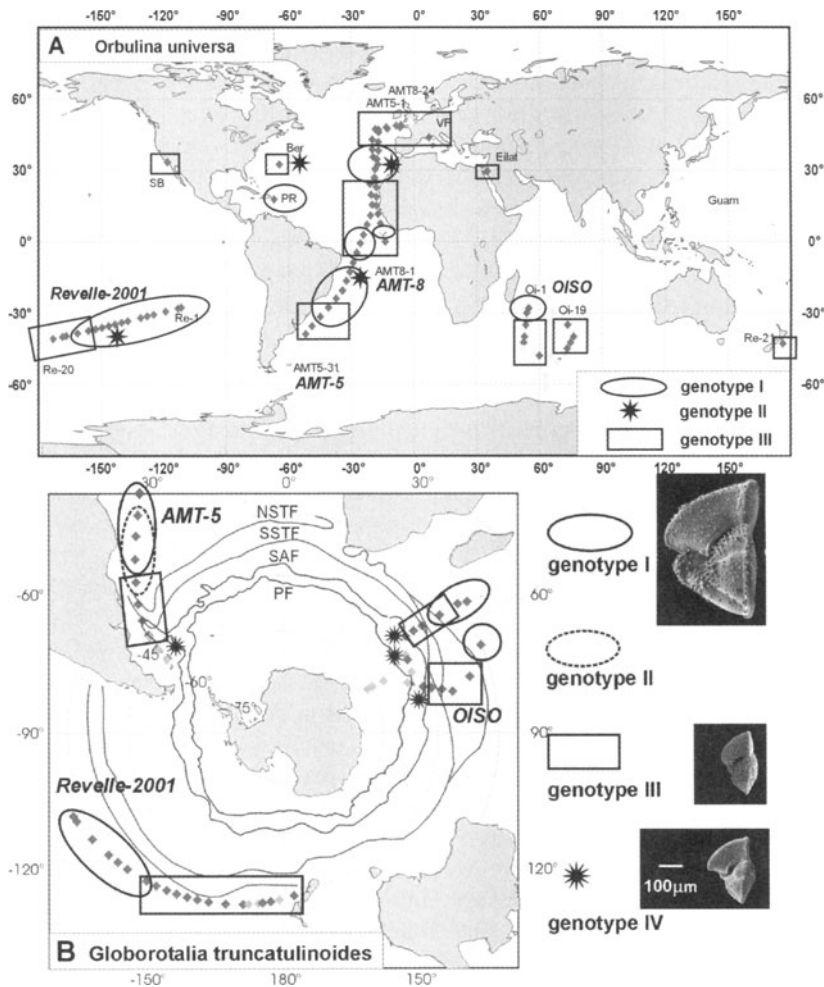


Fig. 3. Molecular biogeography in planktic foraminifera based on single-cell genetic analyses of 102 globally distributed pelagic stations (rhombus, light gray indicate station where the species was absent). **A.** *Orbulina universa*: the genetic provinces are based on the analysis of more than 1200 specimens (de Vargas et al. 1999, unpublished); the sibling species segregate between the vertically mixed and stratified parts of the global ocean (see text). **B.** *Globorotalia truncatulinoides*: 343 specimens have been genotyped along the subtropical to polar ecological gradient in the three main ocean basins (de Vargas et al. 2001, submitted). The four genotypes detected show remarkably predictable biogeographic patterns corresponding to specific latitudinal hydrographic provinces (NSTF = north subtropical front; SSTF = south subtropical front; SAF = sub-antarctic front; PF = polar front). The SEM pictures correspond to specimens from which DNA has been isolated and sequenced.

The diversity and biogeographic patterns of *N. pachyderma* are less clear, possibly because the species complex has been evolving for the past 10–11 million years (Kennett and Srinivasan 1983) over the largest foraminiferal latitudinal range known and displays a high level of morphological complexity. Thus, despite a repeated and intense sampling effort in the sub-polar to polar waters from both hemispheres (more than 500 specimens analyzed, Darling et al. 2001), the lack of data from the tropical to temperate provinces prevents a comprehensive understanding of the diversity and its distribution. In this morphospecies, six different genetic types have been uncovered, which separate into two groups according to the shell's left (L) or right (R) coiling directions (Table 1). According to the SSU rDNA-based phylogeny (Darling et al. 2001), the L and R species-complexes have been genetically isolated since the appearance of the morphospecies 10–11 Ma. Within the "L group", a common ancestor split more than 2 Ma into an endemic arctic species (genotype I), and a group of three species (genotypes II, III, IV) restricted to high latitudes in the southern hemisphere only. In this last group, one species lives south of the polar front in Antarctic polar waters, whereas the two others inhabit the subantarctic domain. The allopatric hydrographic distributions of these southern genotypes may result from successive, relatively recent adaptive steps of speciation towards colder waters, similar to those observed in *G. truncatulinoides*.

Within the "R group", the two sibling species clearly occupy lower latitudes. One genotype was discovered off the Californian coast (Darling et al. 2003), while the other was collected in subpolar waters and is the only component of the *N. pachyderma* species-complex that was found in both hemispheres to date. This last type, together with three genetic types also found in northern and southern subpolar provinces within the *Globigerina bulloides* and *Turborotalia quinqueloba* species complexes (Table 1), were presented as species displaying typical *bipolar* biogeographic distributions (Darling et al. 2000). However, since then, most of these genetic types have also been observed at much lower latitudes in the Santa Barbara Channel and the Canary/Azores currents (Stewart 2000; Darling et al. 2003), and most likely have biogeographic ranges similar to the "transitional vertically mixed" pattern extensively studied in *O. universa* (Fig. 3A).

In fact, the current genetic data suggest that there is not a single bipolar species within the planktic foraminifers. In the four high-latitude species complexes analyzed so far (Darling et al. 2000, 2001; de Vargas et al. 2001), most genotypes have been found in one hemisphere only. The two sister species inhabiting the highest latitudes – the polar genotypes of *N. pachyderma* – have even been separated for more than 2 million years according to the molecular tree (Darling et al. 2001). From the current, still limited data set of planktic foraminifers we may attempt to draw a few new basic distribution rules: (1) species inhabiting the equatorial to temperate/subpolar latitudes are segregated into two major domains, "*transitional vertically mixed*" and "*sub-tropical stratified*" (Fig. 3A). Despite a bipolar tendency, both domains are most probably continuous, in particular, for the "*transitional vertically mixed*" domain, through the cooler eastern boundary currents and the equatorial upwelling zones. (2) Species inhabiting the subpolar to polar latitudes exhibit a high degree of provincialism; their specific allopatric

ranges may typically be organized in relatively narrow latitudinal belts corresponding to different current systems and water masses separated by oceanic fronts (Fig. 3B).

The main distinction between this new molecular biogeography and the classical, morphological one (huge latitudinal, bipolar belts, Bé 1977) consists in the nature of the factors controlling species distributions. Rather than the widely invoked – and in Paleocyanography largely used – sea surface temperatures (Arnold and Parker 1999, Kucera and Darling 2002), biotic factors, such as specific prey and/or symbiont availability, may be the primary elements shaping the distribution of the biological species (de Vargas et al. 2002).

Coccolithophores

In contrast to the planktic foraminifera, the emerging pseudo-cryptic diversity is even less known in coccolithophores, because of restricted sampling (Table 1). A clearly limiting factor here is the need to obtain a sufficient number of cells through clonal culture for DNA extraction and sequencing. However, some biogeographic information can be extracted from previous distribution studies of the morphological variants in deep-sea sediments, sediment traps, or plankton tows, assuming that they correspond to the genotypes. The difficulty to establish a link between genetic and morphological differentiations, and the different morphological species concepts used by different authors render such interpretations hazardous, as illustrated in detail for *C. leptoporus* (Quinn et al. this volume).

Several lines of evidence suggest that the sibling species within a morpho-species are adapted to different spatial and/or temporal allopatric ranges. In two recent analyses of the *C. leptoporus* species complex based on plankton and sediment trap samples, morphologically distinct populations were encountered at specific water depths and seasons (Renaud and Klaas 2001; Renaud et al. 2002). At the Bermuda time-series station the winter populations of *C. leptoporus* were found at a preferred water depth of about 100 m and contained both, intermediate "I" (coccolith diameter 5–8 μm) and large "L" (coccolith diameter $>8 \mu\text{m}$) morphotypes, whereas the summer populations were at 0–25 m water depth and consisted dominantly of morphotype "I". Interestingly, a few intermediate "I" cells covered also with *Crystallolithus rigidus* holococcoliths were observed in May 1991 near the surface and at the transition from large to intermediate forms (Renaud and Klaas 2001). Subsequently, Renaud et al. (2002) could show that the seasonal cell density peaks of *C. leptoporus* "I" also seemed to be variable geographically and occurred at 12°C in spring in the northern North Atlantic (sediment trap NABE, 48°N), at 18°C in spring in the central North Atlantic (sediment trap NABE, 34°N), at 22°C in summer in the western North Atlantic (plankton filters at Bermuda Hydrostation S, 32°N), and at 25°C in fall in the Arabian Sea (sediment trap MST-9, 10°N).

A similar seasonal separation relative to the nutricline-thermocline fluctuation has been observed in the ratio between two morphotypes of *Emiliania huxleyi* (Beaufort and Heussner 2001). In *C. pelagicus*, the large morphogenotype inhabits

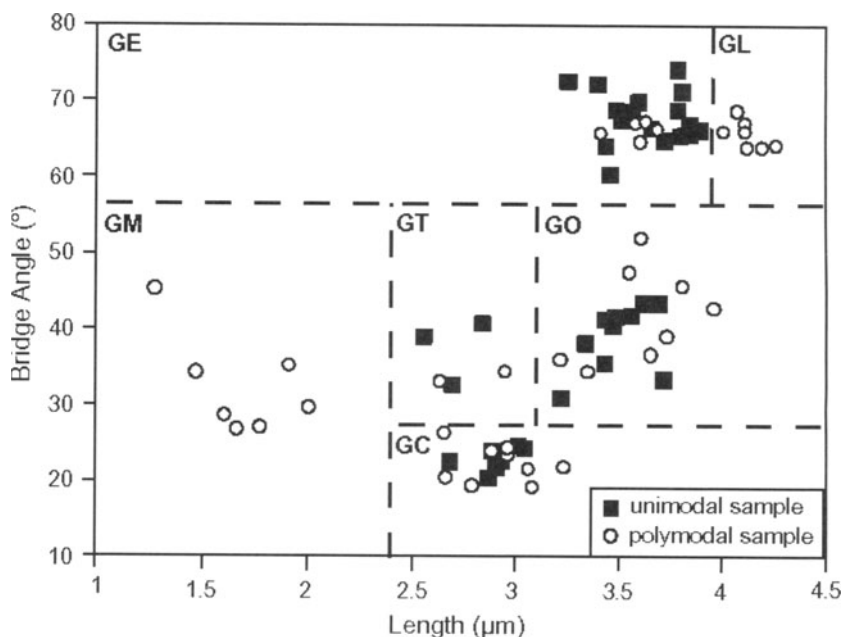


Fig. 4. *Gephyrocapsa* morphotype clusters recognized in 70 globally distributed Holocene assemblages (after Bollmann 1997). GE = *Gephyrocapsa* morphotype Equatorial, GL = Larger, GM = Minute, GT = Transitional, GO = Oligotrophic, GC = Cold.

the northern temperate province, while the small one is adapted to the subarctic water mass (Baumann et al. in prep.).

Although not associated with genetic data, patterns of allopatric ecological ranges between different morphotypes have also been detected in *Gephyrocapsa* sp. In his global Holocene survey, Bollmann (1997) identified six morphotypes based on size and bridge angle. Four of these occurred as unimodal clusters in a total of 39 samples, and an additional two morphotypes could be defined based on distinct clusters in the remaining 31 polymodal assemblages (Fig. 4). The changing morphology was most closely related to surface water temperatures, a relationship that has since been refined to a global paleotemperature transfer function (Bollmann et al. 2002). Although that transfer function is based on the changing frequency of three morphotypes only, the apparent continuity of morphotype clusters in a bivariate plot does indeed allow, if not suggest, the possibility of numerous sibling species adapted to specific temperature niches (Fig. 4). Genetic variability between populations of *G. oceanica* from neritic and oceanic water masses of the NE-Atlantic has previously been inferred from differences in

growth rates observed in cultures of a few isolates from these two water masses (Brand 1982).

Given the current data sets in coccolithophores, it is difficult to say whether the sibling species are more temporally or spatially isolated, and which parameters, abiotic or biotic, are underlying those adaptations. What has become apparent in the few studies done so far, is the presence of distinct morphological subgroupings within many studied species assemblages and the spatial and temporal mobility of these groupings (Knappertsbusch et al. 1997; Bollmann et al. 1998; Knappertsbusch 2000). It is, however, remarkable to notice similarities between the coccolithophore and foraminifer species complexes, such as the shell's size decrease associated with speciation from subtropical/transitional to sub-polar water-masses (Fig. 3B), or the importance of the water column stability as a selective matrix for speciation. There is no *a priori* reason for such parallelism as the two groups, heterotrophs and autotrophs, are biologically fundamentally different (Fig. 1). However, it may be that the spatial/temporal and genetic boundaries between the sibling species correspond to more general community changes, involving intricate and interdependent trophic/parasitic/predatory relationships, which may so far have gone undetected at the morphological level.

Super-species in the plankton – evolutionary and ecological implications

Planktic super-species

As discussed above, the recent application of the molecular tools within pelagic foraminifers and coccolithophores indicates that all classical (morphological) species studied to date are in fact monophyletic clusters of a few sibling, biological species, separated by subtle morphological characters. The sibling species within a morphospecies originated most often many millions of years ago, according to molecular clocks calibrated with the outstanding fossil records of both groups. In addition, global scale, molecular phylogeographic analyses within the foraminifers and morphological biogeographic studies in the coccolithophores, strongly suggest that these sibling species represent geographic or temporal *allopatric* subdivisions of the total range of the traditional morphospecies. Thus, we propose here a new hypothesis for the evolution of the calcareous unicellular plankton, which, we think, may also apply to other holoplanktic organisms. The traditional morpho-taxonomic entities defined as species in foraminifers and coccolithophores, stable over periods of millions years in the fossil record (Thierstein et al. this volume), are in fact *super-species*, i.e. *assemblages of allopatric³ species of monophyletic*

³ In the plankton, *allopatry* does not imply low dispersal. Although the distributions of sibling species within a planktic super-species are largely non-overlapping in space and/or time, there may be mixing at the boundary between two specific ecological ranges, or when individuals of a sibling species are expatriated and drift, as in a resting

origin, often with distinct morphological differences (Mayr 1971, Van der Spoel and Heyman 1983).

The concept of super-species was first introduced to the planktic realm by Fleminger (1972) in his studies of the habitat patterns among the calanoid copepods, where extremely tiny details of the reproductive organs allow accurate discrimination between the biological species. In fact, most other zooplankton morphospecies inhabiting global biogeographic areas with a wide latitudinal coverage, also consist of various morphological variants, each of which occupies a specific ecological subdivision of the total range (Van der Spoel and Heyman 1983), reminiscent of the genetic biogeographic patterns documented in foraminifers (Fig. 3). Depending on their degree of discrimination, those variants are named “varieties” (e.g. the six different latitudinal morphotypes in the Thaliacea *Salpa fusiformis*), or “formae” (e.g. the 5 and 9 morphotypes respectively found in the pteropods *Clio pyramidata* and *Cavolinia tridentata* (Van der Spoel and Heyman 1983). The typical adjoining distribution ranges of the *formae*, separated by contiguous biogeographic boundaries, made that those were interpreted as ecophenotypes within single polytypic species. The present data on foraminifers and coccolithophores strongly suggest that the very common *formae* in pelagic plankton are in fact fully isolated species and thus part of a super-species, which may also be the case for most unicellular pelagic organisms with a cosmopolitan distribution.

A structure-less world

Thus, contrary to widely held views, cosmopolitan, globally distributed unicellular species with high ecological and/or genetic plasticity may be rare or even non-existent in the plankton. Rather, species may be geographically and hydrographically more restricted than previously assumed, and evolve through adaptive speciation linked to the invasion of a new water mass and the distinctive food web it contains.

Moreover, there may be a *significant decoupling* between the important genetic-ecologic and the small morphologic differentiations separating the sibling species within the planktic super-species. This phenomenon of phenotypic uniformity is not exclusive to the planktic *super-species*, but is a general trait of the pelagic life. To paraphrase Verity and Smetacek in their key review on the structure of marine pelagic ecosystems (1996), “among holoplankton, the bulk of the biomass is present within only a few morphotypes ...; in many cases the same basic body shape is maintained through 10x to 100x variation in body volume”. For example, the calanoid copepods encompass more than 1800 species (E. Goetze, pers. com.) within a single basic morphology. Verity and Smetacek explain this morphological constraint by the fact that pelagic evolution of form and function may have been controlled by protection from – or interaction with – other

stage, through the range of another species. When the sibling species display a pattern of depth allopatry at a same geographic location, mixing may also occur due to water diffusion between various layers of the water column.

organisms, rather than competition for resources and resource space as in most terrestrial and, to some extent, marine coastal ecosystems (“watery arms race” hypothesis of Smetacek 2001). Similarly, the dominant role, in an aquatic world, of *chemical* (choice of mate, pheromones, egg-sperm recognition) rather than auditory or visual recognition systems (Knowlton 1993) may have contributed to the restrained structural evolution in the pelagial.

In addition to these “top-down” selective pressures, an essential “bottom-up” evolutionary force must have acted on planktic morphological evolution: the ecological obligation – in order to survive – to float at specific depth(s) in a moving and relatively homogeneous environment. The remarkable profusion of structural convergences between totally unrelated groups of planktic organisms (the basic one being *small size*, Tappan and Loeblich 1973), as well as the striking occurrence of iterative morphological evolution (Cifelli 1969; Norris 1991; Schmidt et al. in prep.), argue in favor of strong environmental pressures shaping the pelagic biosphere. Within the foraminifers and coccolithophores, the relatively heavy calcareous skeleton and the platelets covering the cells may be finely designed and tuned to suit flotation, and the environment may impose a strong *stabilizing selection* on shell shape by rejecting every inappropriate genetic alteration leading to eccentric shapes. In modern foraminifers, this “pelagic selection pressure” on shell design is reflected by the paucity of morphologically defined species in the plankton (about 45), compared to the benthos (about 5000).

Finally, there may be some stabilizing components linked to the unicellular status *per se*. Obviously protists cannot extend their morphological complexity through cellular differentiation like multicellular organisms can. Besides, striking examples of extreme morphological uniformity despite important genetic divergence and species diversity have been described among protists, such as the various isolated mating groups within the ciliate *Tetrahymena pyriformis*, whose phenotypes have persisted unchanged over millions of years (Nanney 1982). The genetic clusters involved in skeleton formation of unicellular organisms may not be so complex in terms of the number of genes and their spatio-temporal networking (Duboule and Wilkins 1998), and thereby also limit the range of potential morphological invention. Whatever the stabilizing selection forces on the phenotype of unicellular plankton may be, they seem strong enough to maintain “optimal phenotypes” through the originations and possibly extinctions of sibling species within a super-species.

Historical patterns – ecological and evolutionary implications

The systematic presence of super-species in planktic foraminifers and coccolithophores suggests that evolution in the pelagial occurs through the creation and extinction of new genetic and ecologic types within a morphologic assemblage, rather than via the expression of phenotypic plasticity within ubiquitous species. This hypothesis fundamentally alters the way we think about pelagic evolution, and has serious implication for the numerous evolutionary, ecological, and paleo-ecologic studies based on the assumption that individuals within morphologically

defined pelagic species share common ecologies. Contrary to these commonly held views, only *minor morphological differences may characterize fully isolated species, which have evolved into radically different environments*.

The global spatio-temporal coverage of a few genetic analyses within the foraminifers (Table 1, Fig. 3) indicates that the number of sibling species within a super-species is *limited*. There are likely between 3–6 times more species than morphologically defined in modern planktic foraminifers, which may make it difficult, but feasible to transfer this biological information to the interpretation of the fossil record. Towards this goal, it is now fundamental to assess the spatio-temporal dynamics and correlations of the genetic, the ecologic, and the morphological variabilities. We propose here two hypothetical endmember models of morphogenetic evolution (A and B in Fig. 5), which may both explain the molecular phylogenetic trees reconstructed within the morphospecies of foraminifers and coccolithophores (Fig. 2).

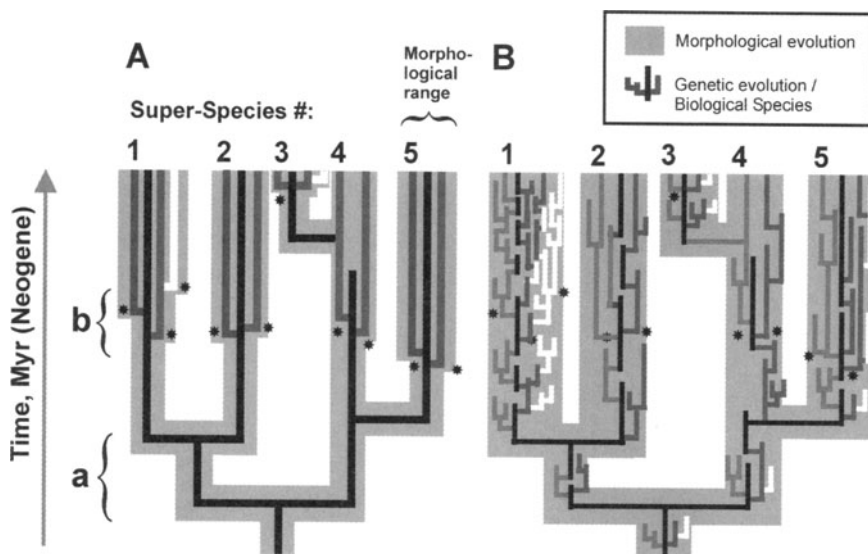


Fig. 5. Two hypothetical models of morphological versus genetic evolution of five planktic super-species within the foraminifers or coccolithophores. The thick lines within the morphological range of each super-species represent the biological species and their stratigraphic extent; different tones symbolize different ecological/biogeographic ranges. An assumed 16 species are living in the modern Ocean. The models A and B illustrate different degrees of disconnection between the morphological, genetic, and ecological differentiations (discussed in the text). Black stars in both trees locate homologous phylogenetic nodes between A and B after which the modern species – or their ancestors in B – started to be genetically fully isolated. Black stars also correspond to the splitting nodes inferred from the molecular trees (Fig. 2).

Model A: Simple spatio-temporal disconnection between the morphological and genetic/ecologic differentiations

The number of sibling species (living and fossil) is not much higher than the number of morphologically defined species. The speciation events leading to the modern species occurred only after the phenotype of the mother species has stabilized into an adaptive, optimal peak and when the super-species increases its ecological range. As depicted in Fig. 5A, the morphological space within a super-species may slightly increase as well during those 'pseudo-cryptic' events of speciation. Biological sibling species are stratigraphically long ranging and they rarely suffer extinction.

Model B: Complex spatio-temporal disconnections between the morphological and genetic/ecologic differentiations

Each morphological species results from an actively ongoing process of originations and extinctions of biological species with different ecological ranges. The sibling species undergo relatively frequent extinctions, but also originate repeatedly from survivor species. The morphological ranges of the super-species have not noticeably changed since their creation.

Current evidence

The current molecular data are not extensive enough, neither with respect to the number of species analyzed nor to the geographic coverage of sampled sites, to decide which of our models, A or B (Fig. 5), better depicts the evolution within the foraminifers and coccolithophores. In foraminifers, the few extensive studies completed so far (de Vargas et al. 1999, 2001, 2002, submitted, Darling et al. 2001), may argue in favor of both models. The evolutionary patterns molecularly revealed within *G. truncatulinoides* for instance (de Vargas et al. 2001), may well match the evolution of *Super-Species 3* of the model B (Fig. 5), while the molecular tree and phylogeographic analyses within *O. universa* (Fig. 2) may better fit model A. Indeed, if the sibling species adapted to different water-masses are relatively old, as in *O. universa* where the phylotypes appeared in the Miocene, model B would imply convoluted evolutionary patterns where the ancestral species of each modern relative within the super-species may display independent and diachronic patterns of iterative adaptive speciation (i.e. Fig. 5B, *Super-Species 1*). If this is the case, it will obviously be much harder to transfer the biological and ecological information extracted from genetic studies of the living species to better interpret the past ecosystems through the study of the fossil record. We could only hope that the past sibling species existed for long enough to evolve unique skeleton phenotypes, or, if they diachronically re-activated similar convergent phenotypes, that similar morphological characters have a common ecological cause.

Taken at their face value the available morphometric data on coccolithophores must also be considered ambiguous (Bollmann 1997; Knappertsbusch 2000). The

morphological evolution of *Calcidiscus leptoporus* could be interpreted, albeit with some generalization, as depicted in model A (Fig. 6). The striking correspondence between the molecular (Fig. 2) and morphological (Fig. 6) data concerning the speciation time – between 10 and 12 Ma – of the “Large” sibling species within *C. leptoporus*, supports our model A.

However, the same data can be interpreted in an alternative way by allowing for a less generalized and more dynamic definition of morphotypes. If the frequency distributions of the ca. 200 coccoliths analyzed in each of the 82 samples from the past 25 million years (Knappertsbusch 2000) are contoured and the resulting uni- to polymodal frequency peaks considered as morphotypes, a

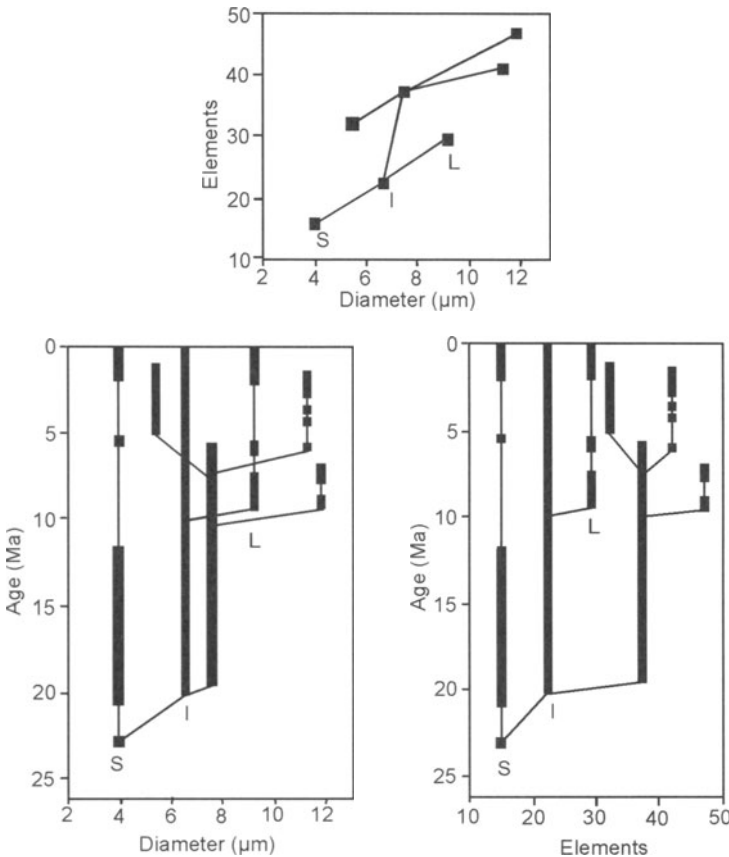


Fig. 6. *Calcidiscus leptoporus*: proposed morphometric phylogenetic tree for a minimum number of seven generalized and stratigraphically static morphotypes (slightly simplified from Knappertsbusch 2000). Thick bars depict reasonably continuous data, thin lines are interpretations. S = *C. leptoporus* small morphotype; I = *C. leptoporus* (Murray and Blackman) Loeblich and Tappan as emended by Sáez et al. 2003); L = *C. quadriperforatus* (Kamptner) Quinn and Geisen (see also Quinn et al. this volume).

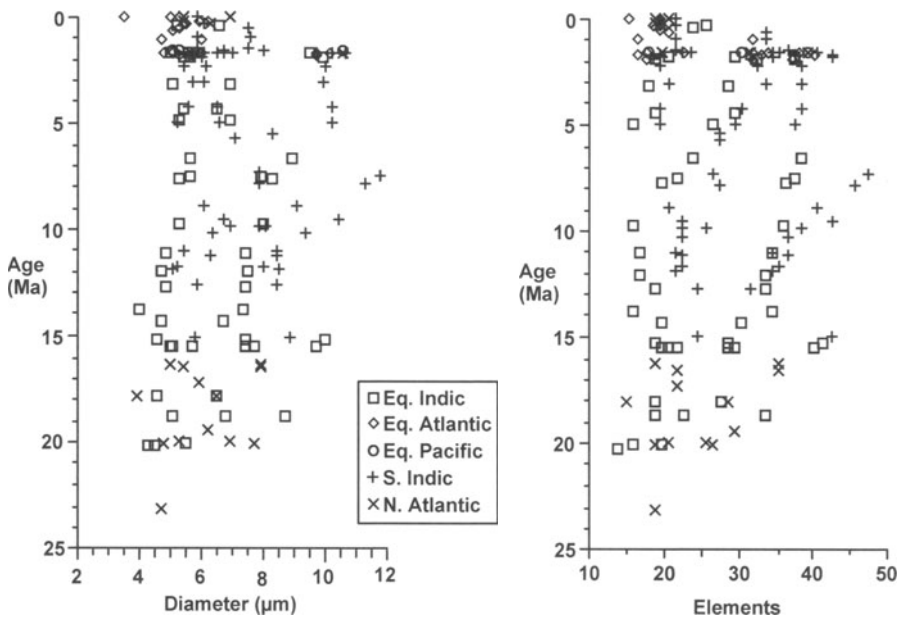


Fig. 7. *Calcidiscus leptoporus*: distribution of the different morphotypes for the last 25 million years, with respect to coccolith diameter (left) and number of elements (right). Here the morphotypes are defined as frequency clusters individually in each of the 82 Neogene samples analyzed from various ocean basins (from Knappertsbusch 1990). In each sample the diameter and the number of elements of about 200 *C. leptoporus* coccoliths were plotted, the resulting frequencies contoured, and the uni- to polymodal frequency peaks considered morphotypes. Eq. = equatorial, S. = South, N. = North.

much more variable pattern of morphologically identifiable populations results (Fig. 7). This alternative interpretation would rather support the evolutionary pattern associated with model B. It is based on a sample by sample analysis of the frequency distribution of morphotypes in bivariate (diameter versus number of elements) plots and shows a much higher spatial and temporal variability of *C. leptoporus* morphotypes than the interpretation using a static morphospecies concept as shown in Fig. 6.

The evidence needed for future distinctions of the occurrence or prevalence of our models A or B in the evolutionary development of the oceanic plankton will have to be a combination of genetic and detailed morphological data. The test will require an inventory of genetic and morphometric variability for the total biogeographic range of living super-species. With that calibration, a new look at the geological record of microfossils, using additional and much more refined morphological criteria, may reveal an evolutionary history much more in tune with evidence from multicellular organisms (see also Thierstein et al. this volume).

Clearly, the super-species approach so far pursued in studies of the deep-sea fossil record has been magnificently successful in late Phanerozoic biostratigraphic dating, where time-resolution has been achieved at the level of a million years or better in most of the Cenozoic (Spencer-Cervato 1999). However, the super-species data resolution may not have revealed the full potential of microfossil applications in paleoecology, paleoceanography, paleoclimatology and evolutionary process studies. We emphasize that the rain of billions of foraminifer and coccolithophore shells to the bottom of the oceans since the Jurassic time produced one of the most precious treasure-troves life left us to understand the co-evolution of organisms, oceans, and climate. The discovery of super-species in plankton groups opens new opportunities for testing evolutionary hypotheses at the interface between biological and paleontological sciences. In addition to further large-scale molecular studies of the modern representatives, the remarkable development of chemical/isotopic paleo-proxies and the promise of new tools to automatically scan, classify and measure microfossils (Bollmann et al. 2002) will allow a factual understanding of the ecological significance of the super-species concept in past oceans. This will also be an essential next step towards a better understanding of pelagic biosphere reactions to climate perturbations, which can now be studied closer to the level at which adaptation and selection operate. Such an approach will also facilitate predictions of how pelagic microbial diversity may respond to the increasingly severe warming events projected in the near future.

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Coccolithophorid biodiversity: evidence from the cosmopolitan species *Calcidiscus leptoporus*

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Summary

Multidisciplinary research on the cosmopolitan coccolithophore species *Calcidiscus leptoporus* within the CODENET research project has revealed that it is composed of at least two separate species (*Calcidiscus leptoporus* and *Calcidiscus quadriperforatus*), characterized by differences in their coccolith morphology, life cycle, distribution, ecology and molecular genetics. This situation, which may be common in the coccolithophores, challenges our view of biodiversity and speciation in this important marine phytoplankton group, at the present day and in the past. Here we present an in-depth review of all available evidence for the existence of hidden evolutionary biodiversity in extant *C. leptoporus*, including new and previously published data from CODENET research.

Introduction

Marine biodiversity is generally underestimated in terms of its scale and importance (Poore and Wilson 1993; Tickell 1997). Whilst current figures indicate that it is only a fraction of that in terrestrial environments (Briggs 1994), marine biodiversity gives us a different perspective compared to that on land, which is crucial for understanding the causes and consequences of biodiversity on a global scale (Williamson 1997). Biodiversity as a concept includes all scales of variability in the natural world (Angel 1997), however it is common to deal with numbers of

species in order to quantify biodiversity (Schluter and Ricklefs 1993). Estimates of species diversity vary considerably depending upon the criterion used to define a species (Williamson 1997). In a strict sense the basic requirement of a species is reproductive isolation (Mayr 1963), however this can be difficult to establish and many studies therefore rely on a morphological definition, or so-called morpho-species concept. Several analyses from marine environments have shown that measurements of biodiversity based upon morphospecies are likely to be underestimated (Knowlton 1993). The commonly occurring microplankton group planktic foraminifera have demonstrated that significant biodiversity, reflecting often very ancient evolution, can be hidden within extant morphospecies (Norris 2000). The occurrence of so-called sibling or cryptic species has indicated that biodiversity and in particular, the significance of very small morphological differences, in this group is poorly understood. Another numerically important, widespread single-celled planktic group, which may display cryptic or sibling species is the coccolithophores. Despite their global distribution and the production of extensive blooms, coccolithophores are represented by only 180 or so species (Jordan and Kleijne 1994). Nevertheless, extensive fine-scale morphological variation has been reported within several commonly occurring coccolithophorid species, including *Calcidiscus leptoporus* (Knappertsbusch 1990; Kleijne 1993; Knappertsbusch et al. 1997), *Gephyrocapsa oceanica*, *Gephyrocapsa muelleriae* and *Gephyrocapsa ericsonii* (Bollmann 1997), *Coccolithus pelagicus* (Baumann 1995; Baumann et al. 2000) and *Emiliania huxleyi* (McIntyre and Bé 1967; Young and Westbroek 1991). These species can be subdivided into separate intra-specific morphological groups, some of which display contrasting geographic distributions in the ocean (Bollmann 1997) and different cell physiologies (Young and Westbroek 1991). Furthermore, significant genetic variability has been demonstrated within the species *E. huxleyi* and *G. oceanica* in terms of their reproductive rates (Brand 1982). New evidence suggests that intra-specific groups are a common occurrence among many extant coccolithophores (Geisen et al. this volume) and as such they are very important for the discussion of hidden biodiversity in this group and the marine plankton as a whole.

In this chapter, we review the degree and nature of biodiversity within one of these species: *C. leptoporus*. Several published and unpublished studies on extant *C. leptoporus* resulting from the CODENET project are reviewed alongside all previous works on the biodiversity of this species (Table 1) and new morphologic data from sediment traps and clonal cultures is presented. The different lines of evidence point to the existence of at least two separate species (*Calcidiscus leptoporus* (Murray and Blackman) Loeblich and Tappan as emended by Sáez et al. 2003 and *Calcidiscus quadriperforatus* (Kamptner) Quinn and Geisen in Sáez et al. 2003), which may have evolved during the last ca. 12 Ma. The picture presented here for extant *C. leptoporus* indicates that the concept of biodiversity in coccolithophores needs to be revised. The findings also have serious implications for the interpretation of morphological variation in the extensive fossil record of coccolithophores, which covers some 200 Ma of geological time (Bown and Young 1998). *Calcidiscus leptoporus* in particular, consists of a succession of several morphological groups during the last ca. 20 Ma (Knappertsbusch 2000).

These may also represent separate biological groups, which have undergone periods of rapid evolution.

Table 1. Comparison of different schemes proposed for the subdivision of living *Calcidiscus*.

Author	Material	Scheme			Comments
Murray and Blackman 1898	Plankton				Indicated two different groups based upon the structure of the central-area
Lohmann 1920	Plankton	<i>Forma parva</i> Sphere: 9–15 μm	<i>Forma typica</i> Sphere: 18–24 μm		
Knappertsbusch 1990	Plankton	<i>Morphotype P</i> Coccoliths: <5 μm <21 elements	<i>Morphotype T</i> Coccoliths: >5 μm >21 elements		
Kleijne 1993	Plankton	<i>Type A</i> Sphere: 6.5–12.5 μm Coccoliths: 3–4.9 μm 15–20 elements. Sutures straight then kinked with incised laevogyre. Extend into central-area	<i>Type C</i> Coccoliths: 4.9–7.2 μm 18–26 elements. Sutures curved or slightly kinked but not incised. Extend into central-area	<i>Type B</i> Coccoliths: 7.5–9.6 μm 27–34 elements. Sutures curved with smooth laevogyre. Not visible in central-area, which is infilled	
Knappertsbusch et al. 1997	Plankton	<i>Small morphotype (S)</i> Sphere: $\leq 10.5 \mu\text{m}$ Coccoliths: $\leq 5 \mu\text{m}$	<i>Intermediate morphotype (I)</i> Sphere: 10.5–16 μm Coccoliths: 5–8 μm	<i>Large morphotype (L)</i> Sphere: $\geq 16 \mu\text{m}$ Coccoliths: $\geq 8 \mu\text{m}$	

Evolution of morphometric concept in *Calcidiscus*

In their initial description of living *C. leptoporus* as *Coccosphaera leptopora*, Murray and Blackman (1898) reported morphological differences between the coccoliths on individual coccospheres of this species from the North Atlantic. With respect to the central-area on the distal shield of *C. leptoporus* coccoliths, they found cells in which the elemental sutures can be traced into this structure and others in which it cannot. This distinction, which was elucidated using a light microscope, was not made in many subsequent analyses on living *C. leptoporus*, but is now considered to be a key feature in distinguishing intra-specific groups within the species.

Lohmann (1920) observed that two size groups occurred in populations of living *C. leptoporus* from the Atlantic Ocean. The large cells, which he called *C. leptoporus* forma *typica*, had a coccosphere diameter of 18–24 μm and the small cells (*C. leptoporus* forma *parva*) were between 9–15 μm (Table 1). He suggested that these two groups had different bathymetric ranges, with *C. leptoporus* forma *parva* occurring in large numbers throughout the entire photic zone and *C. leptoporus* forma *typica* being restricted to shallower water between 0–75 m. Knappertsbusch (1990) subsequently interpreted this bathymetric differentiation in terms of a preference of *C. leptoporus* forma *typica* for warmer water. A further distinction between the two groups was that cells of the smaller forma were found to occur in chains, whereas the larger ones were usually solitary (Lohmann 1920).

During their study of the latitudinal distribution of coccolithophores in the surface waters of the Atlantic Ocean, McIntyre and Bé (1967) discovered large cells occurring in the sub-Antarctic region. These were said to be of “greater size” than the rest of the *C. leptoporus* cells encountered in their study and had an average of 40 elements in the distal shield of their coccoliths (McIntyre and Bé 1967, p. 569). In the absence of definite measurements on the coccosphere diameter of this large group, comparison with the forma of Lohmann (1920) is difficult. However, the observation of large *C. leptoporus* cells associated with cold water conflicts with the ecological interpretation of Lohmann’s (1920) two forma above.

As part of an in-depth study on living and fossil *C. leptoporus*, Knappertsbusch (1990) analyzed the morphological variability of 33 coccospheres from the Atlantic and Pacific Oceans and the Mediterranean Sea. He concluded that the coccoliths on individual coccospheres of *C. leptoporus* were unimodal with respect to size and the number of elements in the distal shield. These two parameters exhibited a strong positive linear correlation ($r = 0.860$). In addition, the coccosphere diameter and mean coccolith diameter of individual cells were also highly correlated ($r = 0.936$), so that one parameter may be used to predict the other.

By analyzing the coccosphere and coccolith morphology of living *C. leptoporus* in this way, Knappertsbusch (1990) confirmed the presence of two size groups, as suggested by Lohmann (1920). The two groups or “morphotypes”, which he called *C. leptoporus* morphotype P and morphotype T (after Lohmann’s

forma *parva* and *typica*), were separated at 10.5 μm coccosphere diameter, 5 μm mean coccolith diameter and a mean of 21 elements in the distal shield (Table 1).

Knappertsbusch (1990) then interpreted the diameter and mean number of elements of *C. leptoporus* coccoliths in 35 globally distributed Holocene surface sediment samples in terms of these two living morphotypes. A plot of the mean number of elements of 200 coccoliths from each Holocene sample against the mean sea surface temperature revealed a shift towards larger coccoliths at 23.5°C. This was considered to be a consequence of a dominance of the large morphotype T in tropical waters above 23.5°C. Knappertsbusch (1990) concluded that this morphotype has a preference for warmer water and the smaller morphotype P is eurythermal, exhibiting a more cosmopolitan distribution.

The morphology of *C. leptoporus* has also been investigated by Kleijne (1991, 1993), from surface water samples collected in the Indian Ocean, Red Sea, Mediterranean Sea and eastern North Atlantic. At one station in the eastern Mediterranean, she discovered a single cell containing heterococcoliths belonging to *C. leptoporus* and holococcoliths corresponding to the species *Crystallolithus rigidus*. Kleijne (1991) interpreted this combination as a transition between two stages in the life cycle of *C. leptoporus*. These two stages were subsequently classified as forma, with *C. leptoporus* forma *leptoporus* representing the heterococcolith stage and *C. leptoporus* forma *rigidus* as the holococcolith-bearing stage.

Based on heterococcolith morphology, Kleijne (1993) considered there to be three intra-specific groups of living *C. leptoporus* (Table 1). She combined morphometric data, such as coccosphere and coccolith diameter and the number of elements, with ultrastructural observations on the distal shield of heterococcoliths to define three "types" of *C. leptoporus* forma *leptoporus*. One important feature distinguishing her three groups is the nature of the central-area on the distal side of coccoliths. This characteristic was first observed by Murray and Blackman (1898) in their original light microscope description of *C. leptoporus*. Kleijne's (1993) type B has an obscured, infilled central-area on the distal side of the coccolith into which the elemental sutures cannot be traced. It is also characterized by having a large coccolith diameter (7.5–9.6 μm) and was found living in water temperatures of 24.6–30.5°C. In the remaining cells observed by Kleijne (1993), the elemental sutures could be traced into the central-area on the distal shield of their coccoliths. However, two additional types were distinguished within these specimens based upon coccolith size and the shape of the sutures on the distal shield. Type A has coccoliths 3.0–4.9 μm in diameter with kinked, incised suture patterns and Type C, which is intermediate in size between types A and B, has more curved, smooth suture patterns. Like the large type B, type A had a restricted distribution and was confined to temperatures of 25.3–30.3°C, whereas the intermediate-sized type C appeared to be more cosmopolitan.

A reassessment of the morphological data of Knappertsbusch (1990), confirmed the presence of three size groups as suggested by Kleijne (1993). Knappertsbusch et al. (1997) redefined the *C. leptoporus* morphotype concept of Knappertsbusch (1990) by measuring the coccosphere diameter and mean coccolith diameter of an additional 63 coccospheres. A plot of these two parameters

produced three size groups, separated at 10.5–16 μm coccosphere diameter and 5–8 μm mean coccolith diameter. These three clusters or morphotypes (small, intermediate and large) are broadly equivalent to Kleijne's (1993) types, in terms of size (Table 1). However, Knappertsbusch et al. (1997) made no distinction between their morphotypes on the basis of the sutures or central-area of the distal shield of the coccoliths.

Using this new scheme, Knappertsbusch et al. (1997) reassessed the coccolith size data of Knappertsbusch (1990) from 35 globally distributed Holocene surface sediment samples. They determined that the mean *C. leptoporus* diameter at different geographical locations was produced by changes in the relative abundance of the three morphotypes. Coccoliths of the large morphotype (>8 μm diameter) were more or less restricted to sediment samples collected within the 23.5°C sea surface winter isotherms, once more suggesting a preference of large *C. leptoporus* cells for warm water. In agreement with Kleijne (1993), the intermediate morphotype was found to have a more cosmopolitan distribution.

New developments from the CODENET project

Sediment trap material

Within the CODENET project, the analysis of *C. leptoporus* coccoliths from sediment trap material has shed more light on the nature of the morphological variation in this group and allowed us to test the concepts developed by Knappertsbusch et al. (1997) and Kleijne (1993).

At the ESTOC station off the Canary Islands, Baumann and Sprengel (2000) have investigated the morphology of *C. leptoporus* from sediment trap material collected during a 13-month period from November 1995 to December 1996. The data of Baumann and Sprengel (2000) confirmed the strong correlation between

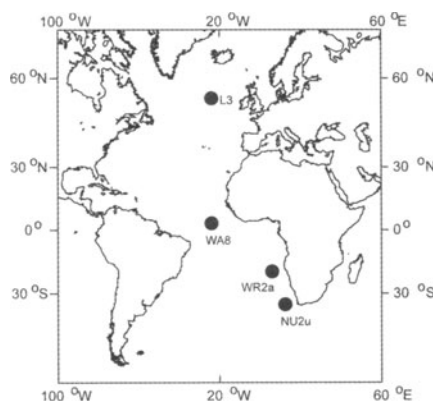


Fig. 1. Location of new sediment trap data analyzed in this study.

C. leptoporus coccolith diameter and the number of elements in the distal shield proposed by Knappertsbusch (1990). By looking at the frequency of coccoliths of a certain diameter over the complete sampling period, three size modes were revealed. These were separated at 5 and 8 μm and correspond well to the three morphotypes/types of Knappertsbusch et al. (1997) and Kleijne (1993). Baumann and Sprengel (2000) also made qualitative observations of the nature of the central-area of the *C. leptoporus* coccoliths they encountered at ESTOC. In this respect, the coccoliths belonging to their large mode had an infilled central-area, while in the intermediate and small sized specimens the sutures of the distal shield could be traced to the center of the coccolith. This observation appears to confirm the findings of Kleijne (1993) in terms of the correspondence between the size and ultra-structure of her three types.

The morphology of isolated *C. leptoporus* coccoliths has also been analyzed from additional sediment trap samples collected at four other sites in the North, Equatorial and South Atlantic (Fig. 1). In all samples, the distal shield diameter and number of elements were recorded for all *C. leptoporus* coccoliths encountered in the monthly samples using an SEM at the Department of Geosciences, University of Bremen (Table 2). As in the study of Baumann and Sprengel (2000), observations were made on the nature of the central-area, in accordance with the descriptions of Kleijne (1993). The distinction between coccoliths with an obscured central-area (type B of Kleijne 1993) and those in which the sutures can be traced into this structure (types A and C) was very clear in this new material. However, the differences in suture patterns between Kleijne's (1993) types A and C were not consistently applicable.

As in the study of Baumann and Sprengel (2000), a scatter plot of diameter and number of elements of all *C. leptoporus* coccoliths measured at all four sites indicates a strong linear relationship ($r = 0.76$) between the two parameters (Fig. 2A).

Table 2. Morphological analysis of *C. leptoporus* coccoliths from four sediment traps in the Atlantic Ocean

Trap	L3	WA8	WR2a	NU2u
Location	N. Atlantic	Eq. Atlantic	S. Atlantic	S. Atlantic
Depth (m)	2200	718	608	2516
Sampling period	8.1995– 6.1996	8.1994– 2.1996	3.1989– 3.1990	1.1992– 2.1993
Coccoliths measured	16	48	77	14
Minimum diameter (μm)	5.7	3.0	3.2	4.6
Maximum diameter (μm)	11.3	8.9	10.0	8.7
Mean diameter (μm)	7.4	5.5	5.6	6.4
SD diameter (μm)	1.6	2.0	1.2	1.0
Minimum no. elements	19	10	10	16
Maximum no. elements	36	31	32	29
Mean elements	24.9	20	17.8	21
SD elements	5.6	6.5	3.8	3.6

SD Standard Deviation, no. Number

By plotting the frequency of coccolith diameter of all specimens measured at all sites, three broad clusters are again visible (Fig. 2B). These correspond quite well to the coccolith size definitions of the morphotypes and types of Knappertsbusch et al. (1997) and Kleijne (1993). However, by analyzing the coccoliths with a clear and an obscured central-area separately, it can be seen that they overlap strongly in terms of size (Figs. 2C and 2D). From this we can conclude that Kleijne's (1993) *C. leptoporus* type B, with its distinctive obscured central-area, is not strictly equivalent to the large morphotype ($>8\ \mu\text{m}$) of Knappertsbusch et al. (1997). The overlap in size between coccoliths with an obscured and a clear central-area can be seen more clearly by highlighting them in a scatter plot of distal shield diameter versus number of elements (Fig. 3A). This also reveals that for a

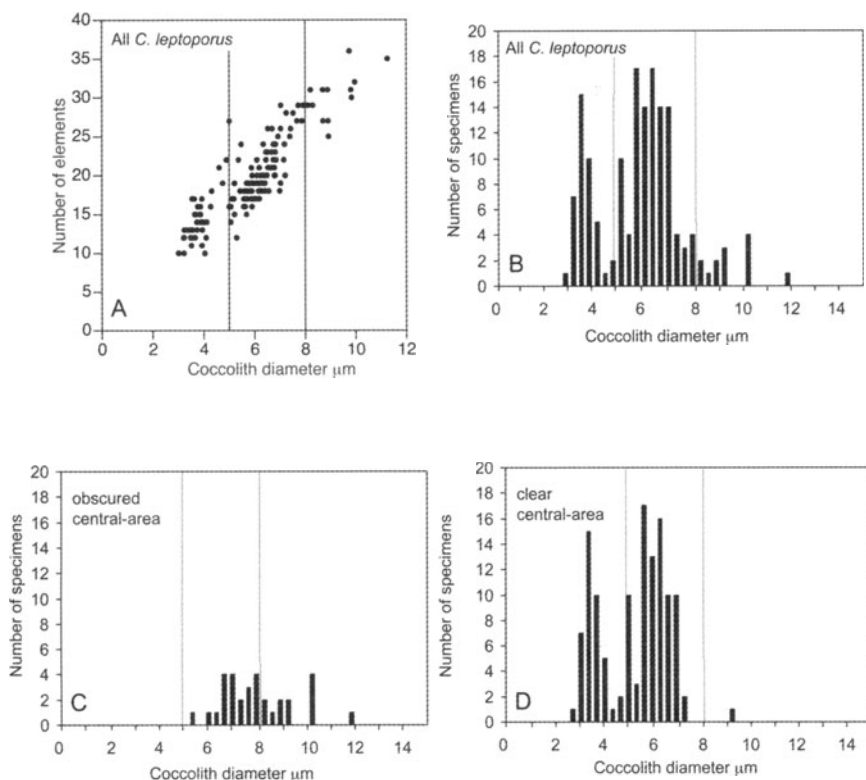


Fig. 2. Morphology of *C. leptoporus* coccoliths from sediment traps. **A.** Scatter plot of diameter and number of elements in the distal shield of all coccoliths analyzed from all sediment traps. **B.** Histogram of coccolith diameter of all coccoliths. **C.** Histogram of coccolith diameter of coccoliths with an obscured central-area on the distal shield. **D.** Histogram of coccolith diameter of coccoliths with a clear central-area on the distal shield. Vertical lines indicate the 5–8 μm boundaries of coccolith diameter proposed by Knappertsbusch et al. (1997) to separate their three *C. leptoporus* morphotypes.

given diameter, coccoliths with an obscured central-area have a greater number of sutures on the distal shield elements (compare Figs. 3B and 3C).

A conspicuous zone of minimum overlap exists in the distribution of coccolith diameter at ca. $5\ \mu\text{m}$. This is in agreement with the occurrence of a small and an intermediate-sized morphotype, as suggested by Knappertsbusch et al. (1997). In the absence of consistent discrimination between the different sutural patterns of Kleijne's (1993) small and intermediate-sized types A and C, it is not possible to compare the size and morphological characteristics of these two groups. However, a small and an intermediate-sized cluster are clearly visible by comparing coccolith diameter and number of elements (Figs. 3C and 3D). These two clusters fall either side of $5\ \mu\text{m}$ in terms of coccolith diameter and have a different coccolith

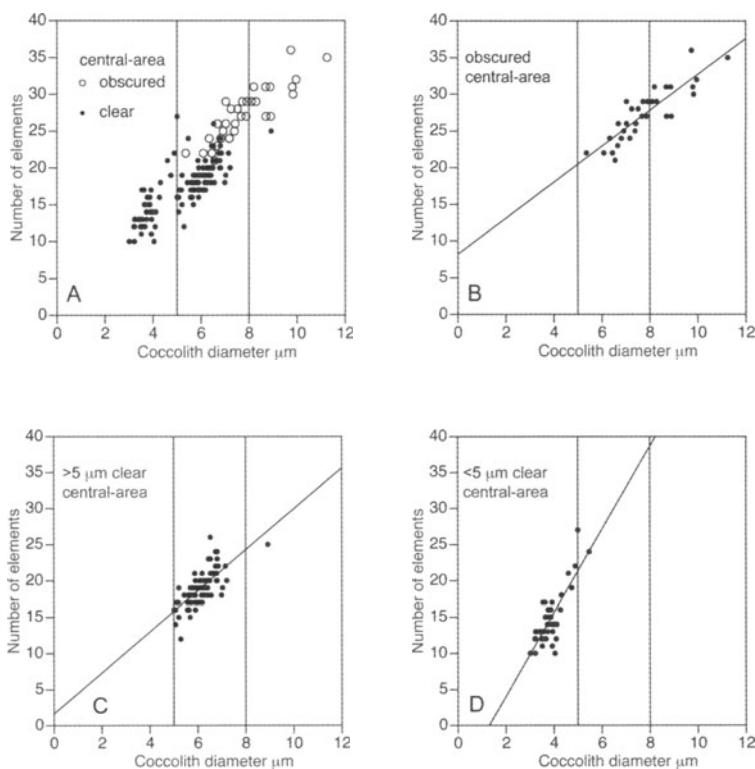


Fig. 3. Morphology of 155 *C. leptoporus* coccoliths from sediment traps. **A.** Scatter plot of diameter and number of elements in the distal shield of all coccoliths analyzed from all sediment traps indicating those coccoliths with an obscured central-area. **B.** Linear correlation between diameter and elements of all coccoliths with an obscured central-area ($r = 0.76$). **C.** Linear correlation of coccoliths $>5\ \mu\text{m}$ with a clear central-area ($r = 0.53$). **D.** Linear correlation of coccoliths $<5\ \mu\text{m}$ with a clear central-area ($r = 0.66$). Vertical lines indicate the $5\text{--}8\ \mu\text{m}$ boundaries of coccolith diameter proposed by Knappertsbusch et al. (1997) to separate their three *C. leptoporus* morphotypes.

diameter/element ratio (compare the slope of the linear correlations in Figs. 3C and 3D).

Whilst the study of Baumann and Sprengel (2000) confirms that three groups of *C. leptoporus* occur in the plankton, the new data presented here suggests that the definition of these is not as simple as previously thought. Contrary to the suggestion of Knappertsbusch et al. (1997), size alone is not a reliable parameter to distinguish the three intra-specific groups, as coccoliths with different distal shield morphologies overlap in terms of size. The distinction between coccoliths with a clear and an obscured central-area on their distal shields appears to be valid. However, those with an obscured central-area have a large size range and cannot be considered to be strictly equivalent to the large *C. leptoporus* morphotype ($>8\ \mu\text{m}$) of Knappertsbusch et al. (1997). The new data presented here also reveals a previously unrecorded difference in the relationship between the diameter and the number of elements in the distal shield of coccoliths with a clear and an obscured central-area (Figs. 3B–D).

The sutural differences between Kleijne's (1993) *C. leptoporus* types A and C are considered to be too subjective for the discrimination of intra-specific groups from isolated coccoliths. However, a small and an intermediate-sized cluster of coccoliths with contrasting relationship between distal shield diameter and number of elements are readily distinguishable in our data. These appear to be equivalent to the small ($<5\ \mu\text{m}$) and intermediate ($5\text{--}8\ \mu\text{m}$) morphotypes of Knappertsbusch et al. (1997) in terms of coccolith diameter.

The discovery of differences in the ratio of coccolith diameter and number of elements between three groups in Fig. 3, contradicts the suggestion of Knappertsbusch (1990) that living *C. leptoporus* forms one continuous "morphocline". In fact, the intra-specific groups, which have been distinguished here, form separate clusters in which the two parameters are well correlated. A similar situation has also been recorded in Holocene sediment samples (Quinn unpublished data).

For the purpose of the following discussion, we use a working scheme based on a combination of size and central-area morphology to describe the three intra-specific groups of *C. leptoporus* above. The three groups are labeled small, intermediate and "large" (S, I and 'L') (Table 3 and Fig. 4). The group *C. leptoporus* 'L' includes cells with coccoliths displaying an obscured, infilled central-area, regardless of their coccosphere diameter, mean coccolith diameter or the mean number of elements in the distal shield of their coccoliths. It is not strictly equivalent to morphotype L of Knappertsbusch et al. (1997) or Kleijne's (1993) type B. A similar morphological concept, which uses a combination of size and central-area structure has been applied within CODENET by Renaud et al. (2002) to study the ecology of *C. leptoporus* morphotypes from sediment trap data (Table 3).

Table 3. (next page) Comparison of different schemes proposed for the subdivision of living *Calcidiscus* in plankton, sediment trap and culture studies within CODENET; including working scheme adopted in this study and revised taxonomy of Sáez et al. (2003).

Table 3.

Author	Material	Scheme			Comments
Baumann and Sprengel 2000	Sediment trap	Coccoliths <5 μm	Coccoliths 5–8 μm	Coccoliths >8.5 μm	Central-area infilled in large-sized morphotype
Renaud and Klaas 2001	Plankton		'Intermediate' subpopulation Sphere: 10.5–16 μm	'Large' subpopulation Sphere: >16 μm	Specimens <10.5 μm not recorded
Renaud et al. 2002	Plankton and sediment trap	<i>Ts</i> Coccoliths <5 μm Serrated sutures, traced into center	<i>Ti</i> Coccoliths 5–8.5 μm Smooth sutures, traced into center	<i>Tl</i> Sutures become obscured towards diffused center	<i>Ti</i> and <i>Tl</i> overlap in size
Geisen et al. 2002	Plankton and culture	<i>C. leptoporus</i> ssp. <i>SMALL</i> Coccoliths 3–5 μm , 10–20 elements. Sutures angular and serrated. Holococcolith stage not yet known.	<i>C. leptoporus</i> ssp. <i>leptoporus</i> Coccoliths 5–8 μm , 15–30 elements. Sutures variable. <i>C. rigidus</i> holococcolith phase	<i>C. leptoporus</i> ssp. <i>quadriperforatus</i> Coccoliths 7–11 μm , 20–35 elements. Sutures smoothly curved with obscured zone around tube. <i>S. quadriperforatus</i> holococcolith phase	Emended taxonomy
Working scheme – this report	Culture and sediment trap	<i>Small</i> Coccoliths usually <5 μm , 10–25 elements. Sutures traced into clear central-area. Holococcolith phase not yet known	<i>Intermediate</i> Coccoliths usually 5–8 μm , 15–30 elements. Sutures traced into clear central-area. <i>C. rigidus</i> holococcolith phase	'Large' Coccoliths usually 6–10 μm , 20–35 elements. Sutures cannot be traced into obscured central-area. <i>S. quadriperforatus</i> holococcolith phase	Intermediate and 'Large' overlap greatly in size, but have proven to be genetically separate
Sáez et al. 2003			= <i>C. leptoporus</i>	= <i>C. quadriperforatus</i>	Raised to species level Small not yet cultured

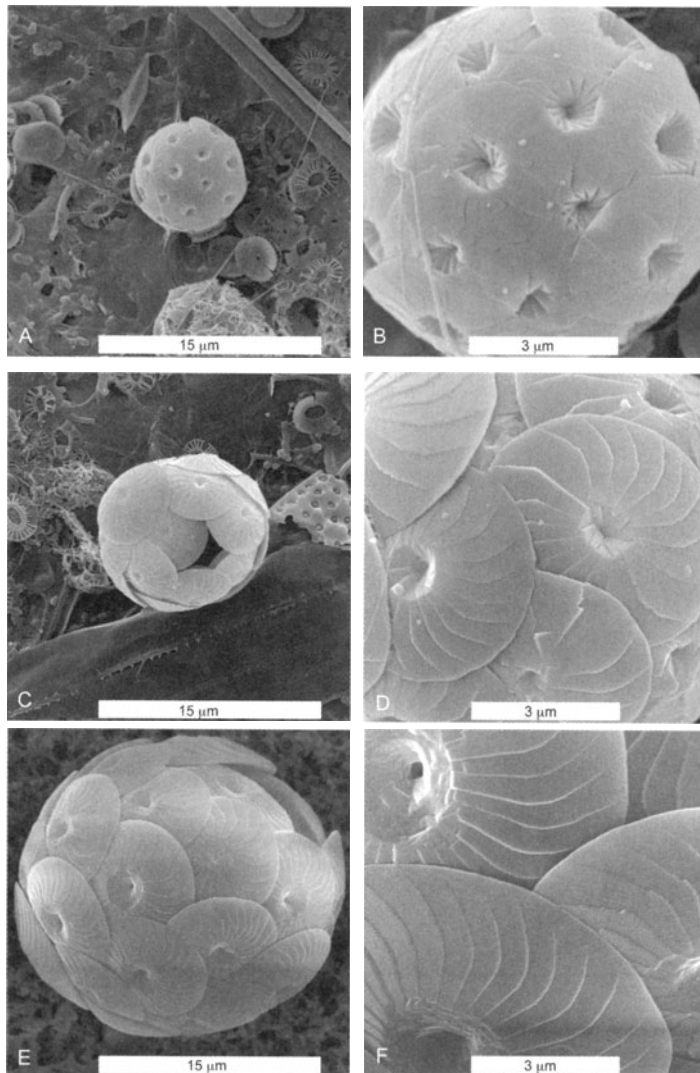


Fig. 4. *C. leptoporus* morphology. **A.–B.** *C. leptoporus* S. Small with a clear central-area, holococcolith stage not yet reported. **C.–D.** *C. leptoporus* I (now *C. leptoporus* (Murray and Blackman) Loeblich and Tappan, as emended by Sáez et al. 2003), intermediate sized with a clear central-area and a *C. rigidus*-bearing holococcolith stage. **E.–F.** *C. leptoporus* 'L' (now *C. quadriperforatus* (Kamptner) Quinn and Geisen in Sáez et al. 2003). Usually large, possessing an obscured central-area, with a *S. quadriperforatus*-bearing holococcolith stage. A–B Meteor Cruise sample FB-27, South Atlantic 20 m, C–F Bermuda Atlantic Time Series (BATS), N. Atlantic 25 m.

Laboratory cultures

The conflicting taxonomic categories to which the various intra-specific groups of *C. leptoporus* have been assigned (Lohmann 1920 – *forma*; McIntyre et al. 1967 – *variants*; Knappertsbusch 1990; Knappertsbusch et al. 1997; Baumann and Sprengel 2000 – *morphotypes*; Kleijne 1993 – *types*) demonstrate the uncertainty surrounding their true biological significance. One hypothesis is that they represent temperature-related ecophenotypes (Knappertsbusch 1990; Kleijne 1993; Knappertsbusch et al. 1997). Knappertsbusch et al. (1997) believed that this idea may be confirmed, “if it could be demonstrated, that clonal descendants of a certain morphotype would change into a different morphotype under varying culturing conditions in the laboratory” (p. 315). Within CODENET, three separate experiments have been carried out to test this hypothesis (Renaud et al. 2000; Steel 2001; Quinn et al. 2003). Two of these experiments (Renaud et al. 2000; Steel 2001) made use of the CODENET culture collection (Probert and Houdan this volume), which is also the subject of ecological and physiological investigations on other common coccolithophorid species.

Clonal populations of *C. leptoporus* I and *C. leptoporus* 'L' cells, were grown in batch culture under different temperature and light conditions (Table 4). In one

Table 4. Summary of CODENET *C. leptoporus* culture experiments

Experiment	Renaud et al. 1999	Steel 2001	Quinn et al. 2003
Group cultured	<i>C. leptoporus</i> I	<i>C. leptoporus</i> I and 'L'	<i>C. leptoporus</i> I
Type of culture	Batch	Batch	Batch
Temperatures (°C)	17, 22	13, 15, 17, 19, 23	16, 28
Light intensity ($\mu\text{E m}^{-2} \text{sec}^{-1}$)	-	Gradient: 30–34	20, 80
Light/dark cycle (hours)	-	16/8	14/10
Duration (days)	-	39	36
Measurement	Coccolith diameter, growth rate	Coccolith diameter, growth rate	Coccosphere and coccolith diameter, mean elements
Outcome	No significant difference between populations grown at 17 and 22°C	No significant difference between populations grown at various temperatures. Different physiological response of two <i>C. leptoporus</i> groups. Holococcolith phase observed	Mean sphere and coccolith diameter slightly smaller at 16°C than at 28°C. Effect of light intensity unclear

experiment (Quinn et al. 2003), a significant but small size difference was recorded between the coccosphere and coccolith size of *C. leptoporus* I maintained under warm (28°C) and cool (16°C) conditions (Fig. 5). Cool clones were less than 3 μm smaller than warm clones and the populations remained within the size limits reported for this infra-specific group. Observations of distal shield morphology throughout the experiment revealed coccolith ultrastructure to be stable under different environmental conditions. In the other two experiments, no significant size or morphological changes were reported in the *C. leptoporus* populations.

The results of these three independent experiments do not support the existence of ecophenotypes. This is in agreement with the rejection of the morphocline hypothesis of Knappertsbusch (1990) above. An interesting outcome in one of the experiments was the observation of a possible physiological difference between *C. leptoporus* I and *C. leptoporus* 'L' populations (Steel 2001). The latter grew at a more restricted temperature range (13–23°C) than the *C. leptoporus* I populations (15–23°C). Both experienced their optimum growth at a temperature of 19°C, but differed in terms of the rate of cell division: *C. leptoporus* 'L' underwent 0.36 divisions per day and *C. leptoporus* I had 0.5 divisions per day. Whilst these differences are small and could represent the unpredictable outcome characteristic of laboratory experiments on living organisms, they are in agreement with the hypothesis of Kleijne (1993) that *C. leptoporus* 'L' cells have a more restricted geographical range, which may be correlated with water temperature.

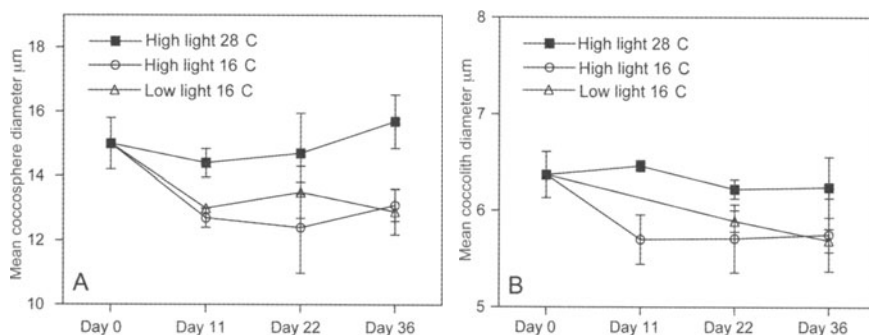


Fig. 5. Ecophenotypic size change in monoclonal *C. leptoporus* cultures (Quinn et al. 2003). **A.** Average mean coccosphere diameter of four clonal *C. leptoporus* I strains maintained under different environmental conditions for 36 days. **B.** Average mean coccolith diameter of four clonal *C. leptoporus* I strains maintained under different environmental conditions for 36 days. For details of experiments see Table 4.

Ecology and seasonality

The body of data on the distribution and ecology of intra-specific groups of *C. leptoporus* is confusing. Confusion has arisen due to different sample materials (plankton, sediment trap, surface sediments) and conflicting definitions of the groups. We can however be certain that the intra-specific groups do exhibit some geographic and temporal differences, which may reflect differences in their ecology. Several studies indicate that larger cells of *C. leptoporus* are restricted to tropical and subtropical latitudes (McIntyre et al. 1970; Knappertsbusch 1990; Kleijne 1993; Knappertsbusch et al. 1997), although their exact geographical limits have been variously defined. Knappertsbusch et al. (1997) have suggested that a possible explanation for this distribution may be a preference of their large morphotype for warm waters. This may also explain the observation of Lohmann (1920), who recorded bathymetric differences between his two forma, with the smaller forma *parva* occurring throughout the photic zone and the larger forma *typica* restricted to shallower water.

Plankton data from the Hydrostation S time series near Bermuda analyzed within CODNET seems to contradict this simplified view of *C. leptoporus* ecology (Renaud and Klaas 2001). In monthly plankton samples collected during 1991–1994, Renaud and Klaas (2001) recorded an intermediate (10.5–16 μm) and a large-sized (<16 μm) subpopulation, which are equivalent to the intermediate and large morphotypes of Knappertsbusch et al. (1997). Specimens <10.5 μm in diameter or with coccoliths <5 μm were not encountered, suggesting that the small morphotype of Knappertsbusch et al. (1997) did not occur at this site during the sampling period. The analysis of monthly samples from various water depths during 1991 failed to confirm the observation of Lohmann (1920) that large *C. leptoporus* cells are restricted to shallow water. In fact, both morphotypes, or subpopulations occurred throughout the water column for a large part of the year. For the two subpopulations, a similar pattern of seasonal dynamics was recorded during the years 1991, 1992 and 1993, in which the large subpopulation only occurred in winter, whereas intermediate cells were present all year round and peaked in late spring. This pattern contradicts the interpretation of Knappertsbusch et al. (1997) that large *C. leptoporus* cells have an ecological preference for warm water. The situation seems to be more complex and may be related to interactions within the plankton community (Renaud and Klaas 2001) or variations in the thermo-nutricline (Beaufort and Heussner 2001).

The *C. leptoporus* data of Renaud and Klaas (2001) from Bermuda has been re-interpreted by Renaud et al. (2002) using a morphological concept comparable to the working scheme adopted here (Table 3), taking into account the details of the central-area of coccoliths in accordance with the observations of Kleijne (1993). The absolute and relative abundance of three morphotypes (Ts, Ti and Tl), equivalent to S, I and 'L' here, was analyzed at Hydrostation S and three sediment traps in the North Atlantic and Arabian Sea. Seasonal patterns in the abundance of the groups differed between the sites, perhaps due to their contrasting oceanographic settings. However, comparisons between the relative abundance of the

three groups at all sites and several environmental variables, suggest differences in their ecology. Whilst both *C. leptoporus* I and *C. leptoporus* 'L' displayed a general increase in absolute abundance with decreasing temperature, the relative abundance of *C. leptoporus* 'L' was greater at higher temperatures and nutrient conditions, and vice versa for *C. leptoporus* I. Renaud et al. (2002) have suggested that *C. leptoporus* 'L' is an opportunistic form, following the dynamics of the total flora. This interpretation could perhaps explain the occurrence of a peak of large cells in winter at Bermuda (Renaud and Klaas 2001) when mixing increases the availability of nutrients.

Life cycle observations

Coinciding with a peak in the abundance of *C. leptoporus* I in late spring 1991 at Hydrostation S near Bermuda was the occurrence of hetero-holococcolith combination cells (Renaud and Klaas 2001). *C. leptoporus* combination cells (*C. leptoporus* I and *C. rigidus*) were first reported by Kleijne (1991) and are thought to represent a transition in a complex haploid-diploid life cycle (Geisen et al. 2002). Similar associations have been discovered for other extant coccolithophores (Cros et al. 2000; Cortés and Bollmann 2002). Cortés (2000) also reported the association of *C. leptoporus* I and *C. rigidus* in May at Bermuda. In addition, she figured a single specimen in which this holococcolith occurs in association with *C. leptoporus* S heterococcoliths (Cortés 2000, p. 36, plate I, Fig. 1).

In the experiments of Steel (2001) the transformation between *C. leptoporus* I hetero- and *C. rigidus* holococcoliths was observed in culture for the first time. Interestingly, this transformation took place at the same time of year as the transition recorded by Renaud and Klaas (2001) at Hydrostation S. Furthermore, an identical, synchronous transformation also took place in two *C. leptoporus* I cultures in the CODENET culture collection (Caen, France) during late spring 2001. Both laboratory cultures were kept under artificial light/dark regimes, suggesting that the life cycle change may have been triggered by an internal biological rhythm.

Recent CODENET plankton observations have also revealed a different holococcolith-heterococcolith combination for *C. leptoporus* 'L' with *Syracolithus quadriperforatus* (Geisen et al. 2000). This observation indicates that *C. leptoporus* I and *C. leptoporus* 'L' cells may differ in terms of the structure of their complex life cycles (Geisen et al. 2002).

Molecular genetics

The subject of genetic biodiversity in living coccolithophores was first addressed by Brand (1982). Based on the acclimatized reproductive rates of *E. huxleyi* and *G. oceanica* cultures, he inferred that significant genetic differences existed between populations of these two species from different water masses. Subsequently,

the first molecular genetic analyses of coccolithophores were carried out by Medlin et al. (1996). This study failed to discriminate between the intra-specific groups of *E. huxleyi* based on the DNA sequences examined. However, analysis of genome size from laboratory cultures confirmed the separation of *E. huxleyi* types A and B of Young and Westbroek (1991). As part of the CODENET project, molecular genetic techniques have been applied to the haptophytes to interpret the evolutionary history of many calcifying and non-calcifying species. To determine the genetic significance of intra-specific groups within *C. leptoporus* (and other species), the plastid gene *TufA* and the more conservative 18S ribosomal DNA gene, have been analyzed by Sáez et al. (2003) from 13 clonal cultures from different geographic regions (Fig. 6).

From the CODENET culture collection, all populations of *C. leptoporus* were analyzed for their number of elements, coccolith diameter and the nature of the central-area. From the available cultures, eight *C. leptoporus* 'L' populations and five *C. leptoporus* I populations were chosen for genetic analysis. No clones of *C. leptoporus* S were available for analysis as this group has not yet been successfully maintained in culture.

Sáez et al. (2003) found that these two groups of *C. leptoporus* cultures had separate *TufA* genotypes, differing by 64–67 nucleotide substitutions. All *C. leptoporus* I populations were identical to each other in terms of the *TufA* gene. However, of the eight *C. leptoporus* 'L' cultures, three with a very small mean diameter (referred to as *C. leptoporus** or L* by Sáez et al. 2003) (Fig. 7), were separable by three substitutions from the five other cultures on the basis of *TufA*, and both subgroups were identical among themselves. For the slower evolving 18S rDNA gene, all eight *C. leptoporus* 'L' populations were identical, but differed from the *C. leptoporus* I populations by four substitutions. The strains belonging to the *C. leptoporus* I group were also identical in terms of 18S rDNA.

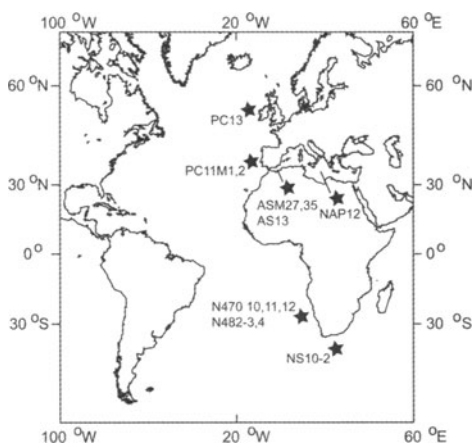


Fig. 6. Origin of clonal *C. leptoporus* I and 'L' cultures from the CODENET culture collection analyzed morphologically in this study and used for molecular genetic analysis by Sáez et al. (2003).

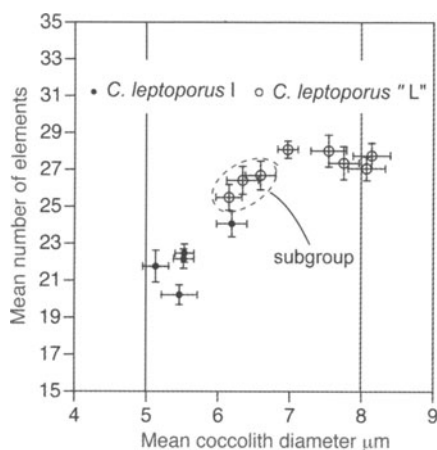


Fig. 7. Morphology of clonal *C. leptoporus* I and 'L' cultures from the CODENET culture collection analyzed morphologically in this study and used for molecular genetic analysis by Sáez et al. (2003). Plot of mean diameter and mean number of elements in the distal shield of coccoliths from five *C. leptoporus* I and eight *C. leptoporus* 'L' coccospheres. The genetic subgroup of *C. leptoporus* 'L' strains (referred to as *C. leptoporus** or L* by Sáez et al. 2003) is indicated. Vertical lines indicate the 5–8 μm boundaries of coccolith diameter proposed by Knappertsbusch et al. (1997) to separate their three *C. leptoporus* morphotypes. Error bars indicate $\pm 95\%$ confidence intervals for the means.

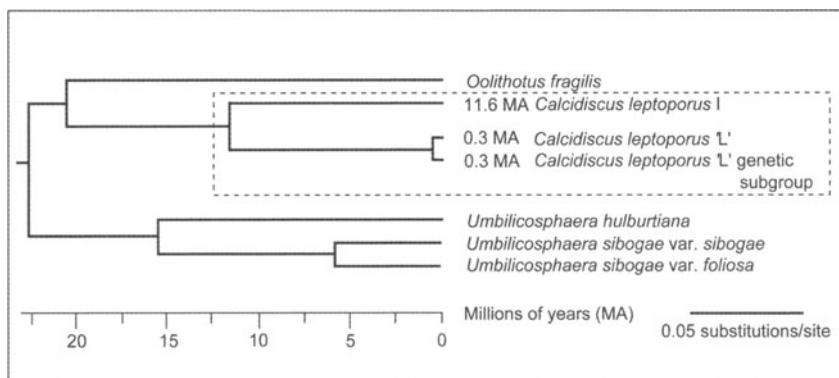


Fig. 8. Molecular phylogenetic relationships in *C. leptoporus*. Linearised TufA tree for *C. leptoporus* and related species from Sáez et al. (2003). Age estimations for the three lineages of *C. leptoporus* were calibrated using 23 Ma for the appearance of *Calcidiscus* and *Umbilicosphaera* (de Kaenel and Villa 1996; Young 1998). Tree redrawn from Sáez et al. (2003). Genetic subgroup of *C. leptoporus* 'L' referred to as *C. leptoporus** or L* by Sáez et al. (2003). *Calcidiscus leptoporus* I now *C. leptoporus* (Murray and Blackman) Loeblich and Tappan, as emended by Sáez et al. 2003, *C. leptoporus* 'L' now *C. quadriperforatus* (Kamptner) Quinn and Geisen in Sáez et al. 2003.

The 18s rDNA and TufA molecular results indicate that *C. leptoporus* I and *C. leptoporus* 'L' are genetically separate. The *C. leptoporus* TufA gene also suggests the presence of a third group, closely related genetically and morphologically to the isolates from the *C. leptoporus* 'L' group. This third group has not been previously identified. The mean coccolith size difference between these three populations and that of the other five *C. leptoporus* 'L' cultures is very small (Fig. 7). Further studies are therefore needed to establish the biological significance of these two *C. leptoporus* 'L' groups and identify additional morphological criteria to separate them.

Table 5. Morphological analysis of 13 clonal populations of *C. leptoporus* from the CODENET culture collection

Clone		Analysis							
Code	Origin	ID	N	Diameter (μm)		Mean	SD	CI	
				Min.	Max.				
N470/10	S. Atlantic	I	30	4.8	6.3	5.5	0.4	0.1	
N470/11	S. Atlantic	I	30	4.2	6.1	5.1	0.5	0.2	
N470/12	S. Atlantic	I	30	4.6	6.1	5.5	0.4	0.1	
AS31	W. Med.	I	30	5.0	7.5	6.2	0.6	0.2	
NS10/2	S. Atlantic	I	30	4.6	6.4	5.5	0.4	0.1	
PC11M1	N. Atlantic	'L'	30	5.3	7.0	6.2	0.5	0.2	
PC11M3	N. Atlantic	'L'	30	5.5	8.2	6.3	0.6	0.2	
PC13	N. Atlantic	'L'	30	5.2	7.9	6.6	0.6	0.2	
ASM27	S. Atlantic	'L'	30	7.0	9.2	7.8	0.6	0.2	
N482/3	S. Atlantic	'L'	30	7.0	10	8.1	0.7	0.3	
N482/4	S. Atlantic	'L'	30	6.8	9.6	8.1	0.7	0.3	
ASM35	W. Med.	'L'	30	6.3	9.1	7.5	0.7	0.3	
NAP12	W. Med.	'L'	30	5.8	8.3	7.0	0.7	0.3	
Code	Origin	ID	N	Elements		Mean	SD	CI	
				Min.	Max.				
N470/10	S. Atlantic	I	30	20	25	22.2	1.4	0.5	
N470/11	S. Atlantic	I	30	17	25	21.8	2.4	0.9	
N470/12	S. Atlantic	I	30	19	25	22.5	1.4	0.5	
AS31	W. Med.	I	30	20	28	24.1	2.0	0.7	
NS10/2	S. Atlantic	I	30	18	23	20.2	1.3	0.5	
PC11M1	N. Atlantic	'L'	30	21	29	25.5	1.9	0.7	
PC11M3	N. Atlantic	'L'	30	22	31	26.4	2.1	0.8	
PC13	N. Atlantic	'L'	30	23	31	26.7	2.2	0.8	
ASM27	S. Atlantic	'L'	30	23	33	27.4	2.5	0.9	
N482/3	S. Atlantic	'L'	30	24	32	27.8	1.9	0.7	
N482/4	S. Atlantic	'L'	30	24	31	27.1	1.8	0.6	
ASM35	W. Med.	'L'	30	23	34	28.0	2.4	0.9	
NAP12	W. Med.	'L'	30	25	31	28.1	1.5	0.5	

ID. *C. leptoporus* group, I *C. leptoporus* I (*C. leptoporus* (Murray and Blackman) Loeblich and Tappan, as emended by Sáez et al. 2003), 'L' *C. leptoporus* 'L' (*C. quadriperforatus* (Kamptner) Quinn and Geisen in Sáez et al. 2003), N, number of coccoliths measured, SD, standard deviation, CI, 95% confidence interval of means

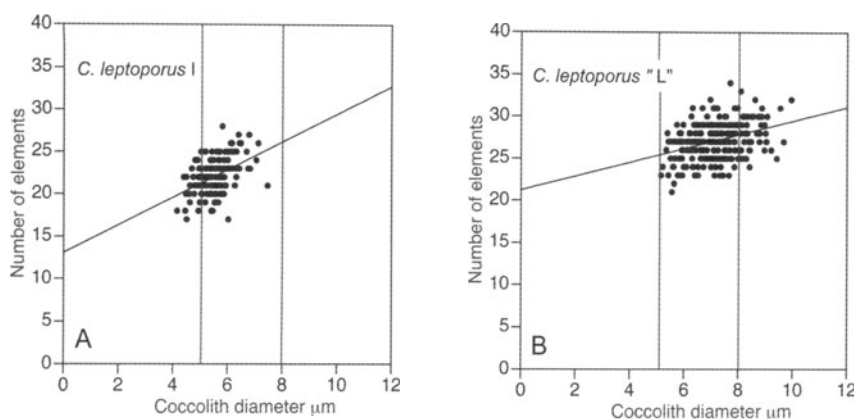


Fig. 9. Morphology of *C. leptoporus* I and 'L' culture strains from CODENET culture collection analyzed morphologically in this study and used for molecular genetic analysis by Sáez et al. (2003). **A.** Linear correlation between diameter and elements of coccoliths from five *C. leptoporus* I strains. **B.** Linear correlation between diameter and elements of coccoliths from eight *C. leptoporus* 'L'. Vertical lines indicate the 5–8 μm boundaries of coccolith diameter proposed by Knappertsbusch et al. (1997) to separate their three *C. leptoporus* morphotypes. 30 coccoliths measured from each culture strain – A. $n = 150$, B. $n = 240$.

Molecular clock calibration using 23 Ma for the emergence of the genus *Calcidiscus* and *Umbilicosphaera* (de Kaenel and Villa 1996; Young 1998), suggests that the *C. leptoporus* I and *C. leptoporus* 'L' lineages originated in the Middle Miocene at about 11.57 ± 1.61 Ma (Fig. 8). The genetically distinct subgroup of *C. leptoporus* 'L' populations may be part of a separate lineage that appeared in the Late Pleistocene at 0.32 ± 0.19 Ma (Fig. 8).

New morphological analysis of the CODENET *C. leptoporus* populations analyzed genetically by Sáez et al. (2003) supports the new sediment trap coccolith data presented above. In each culture, the diameter and number of elements were recorded for 30 coccoliths from intact coccospheres (Table 5). A comparison of the diameter and mean elements of coccoliths from all *C. leptoporus* I and *C. leptoporus* 'L' populations analyzed, confirms that there is a strong overlap between the two morphotypes in terms of coccolith size (Fig. 9). Most of the *C. leptoporus* 'L' populations had a mean coccolith diameter of $<8 \mu\text{m}$ (Table 5) and were at the lower end of the size range reported for this group in the sediment trap data. Similarly, some cells of the *C. leptoporus* I populations analyzed possessed individual coccoliths $<5 \mu\text{m}$ in diameter (Fig. 9A), however the mean of all individual populations was $>5 \mu\text{m}$ (Table 5).

A comparison of the coccolith diameter and number of elements of all coccoliths from each group of *C. leptoporus* cultures also supports the suggestion that they exhibit a different relationship between these two parameters (compare the linear correlations in Figs. 9A and 9B). These two scatter plots reveal that there is a large variation in terms of coccolith diameter and number of elements in the

clonal laboratory populations. In some of the cultures analyzed, the coccoliths ranged from perfectly formed to badly malformed, however care was taken to analyze only those coccoliths that were well formed. It therefore appears that the morphology of *C. leptoporus* is less stable when grown under artificial, laboratory conditions compared to wild populations (compare Fig. 9 with Fig. 3).

Discussion and conclusions

Biodiversity in extant *Calcidiscus*

Significant morphological, ecological and genetic biodiversity occurs in the extant coccolithophore *C. leptoporus*. Much of this variability has been known for some time. However, early attempts to describe it have differed in terms of the number of intra-specific groups, the criteria used to define them and opinions surrounding their biological significance. By reviewing several lines of evidence resulting from the CODENET project in the context of all previous research on the nature of extant *C. leptoporus*, we conclude that the intra-specific variation within this coccolithophore is not as easy to define as previously thought. Nevertheless, by combining biometric data with qualitative observations on the coccolith ultrastructure in extant *C. leptoporus*, three morphological groups can be seen, which differ in terms of their geographic distribution, ecological preferences and perhaps life cycles. Furthermore, molecular genetic analysis of extant *C. leptoporus* has confirmed the existence of two of these three and demonstrated that they are separate biological groups, which evolved as independent lineages ca. 12 Ma ago. We predict that the small morphotype will also be genetically distinct given the amount of divergence found between the other morphotypes.

Taxonomy and biological significance

A recent first attempt at formally amending the taxonomy of *C. leptoporus* was made by Geisen et al. (2002) (Table 3). Based on the groups described above, this species was subdivided into three subspecies: *C. leptoporus* ssp. *leptoporus* (intermediate-sized with a clear central-area and with a *C. rigidus*-bearing holococcolith stage – *C. leptoporus* I in the above discussion), *C. leptoporus* ssp. *quadriperforatus* (usually large in size, possessing an infilled central-area, with a *S. quadriperforatus*-bearing holococcolith stage – *C. leptoporus* 'L') and *C. leptoporus* ssp. SMALL (small sized with a clear central-area, holococcolith stage as yet unreported – *C. leptoporus* S). In accordance with the concept of Cros et al. (2000), a single taxonomic name was used for both hetero- and holococcolith phases of this coccolith. Cros et al. (2000) proposed that the non-Linnaean suffix HO should be added to denote the holococcolith-bearing phase of species in which a complex life cycle has been demonstrated. This is preferable to the use of sepa-

rate forma, as proposed by Kleijne (1991). Forma, the lowest category of botanical nomenclature is used to describe minor variations within a population (Lincoln et al. 1982), and as such is not appropriate for the description of life cycle phases within a single species.

According to Mayr (1963), the basic requirement for a species is reproductive isolation. Subspecies on the other hand are capable of interbreeding, yet may possess minor genetic differences. In the case of phytoplankton, Knappertsbusch (1990, p. 82) extended Mayr's (1970) definition of a subspecies by stating that they inhabit "a geographic, bathymetric and ecological subdivision of the range of the species". This is in agreement with the different distributions and ecologies suggested for the three groups of *C. leptoporus*. However, species also differ in terms of their ecological, geographic and bathymetric limits (Winter et al. 1994), in which case, tests are required to determine whether the different groups of *C. leptoporus* can interbreed. Nevertheless, the genetic analyses of Sáez et al. (2003) suggest *C. leptoporus* I and 'L' have been reproductively isolated for ca. 11.6 Ma. This conclusion was based on the absence of genetic variation in TufA and 18S rDNA within each group (with the exception of the small subgroup of *C. leptoporus* 'L') and the genetic differences between the two in terms of these two genes. If *C. leptoporus* I and 'L' were interbreeding and had exchanged genetic material by recombination (18S rDNA) and chromosomal segregation (TufA), then such close correspondence between morphology and genetics would not be expected. Based on this molecular evidence from TufA and 18S rDNA, Sáez et al. (2003) have therefore raised the subspecies *Calcidiscus leptoporus* ssp. *leptoporus* and *Calcidiscus leptoporus* ssp. *quadriperforatus* of Geisen et al. (2002) to full species rank, as *Calcidiscus leptoporus* (Murray and Blackman) Loeblich and Tappan and *Calcidiscus quadriperforatus* (Kamptner) Quinn and Geisen in Sáez et al. 2003, respectively.

Biodiversity in coccolithophores

The review presented here demonstrates that wide biodiversity occurs on several levels within the extant coccolithophore *Calcidiscus*. This can be most appropriately defined by the presence of three morphological groups, two of which have been proven to be separate species. Studies of several other taxa, including *Gephyrocapsa oceanica*, *Gephyrocapsa muelleriae* and *Gephyrocapsa ericsonii* (Bollmann 1997), *Coccolithus pelagicus* (Baumann 1995; Baumann et al. 2000) and *Emiliania huxleyi* (McIntyre and Bé 1967; Young and Westbroek 1991) have also revealed the existence of intra-specific variation and it appears that this may be a common picture within the coccolithophores (Geisen et al. this volume). It is clear that significant biodiversity may be revealed by the detailed analysis of single taxa of coccolithophores, which is not accounted for in the current morphospecies concepts. As can be seen in extant *Calcidiscus*, much of this variation is genotypic in origin and what has been described as a single biological group may in fact be a plexus of closely related species. In the only other genetic studies on coccolithophores published to date, Medlin et al. (1996) and Iglesias-Rodriguez et al. (2002)

have revealed that two of the three intra-specific groups of living *E. huxleyi* can also be distinguished on the basis of their DNA, suggesting that they may also reflect genotypic variation within this taxon.

If the separate genetic groups within *Calcidiscus* and other coccolithophores such as *E. huxleyi* are indeed separate species, then the picture of biodiversity in terms of species richness needs revising. However, the documentation of combination cells containing different hetero- and holococcoliths (Kleijne 1991; Cortés 2000; Cros et al. 2000; Cortés and Bollmann 2002; Geisen et al. 2002) indicates that many coccolithophores classified as separate morphospecies may in fact be different stages in the life cycle of the same organism.

The picture of coccolithophore biodiversity in *Calcidiscus* and other common coccolithophores has serious implications for the interpretation of fossil coccolith morphology and the assessment of species diversity during the 200 Ma-history of these organisms (Bown et al. this volume). Given the low preservation potential of holococcolithophores (Bown and Young 1998), the fossil record of coccolithophores is dominated by heterococcoliths. Fossil heterococcolith taxonomy is based solely upon the information contained within coccolith morphology and crystallography (Bown and Young 1997; Young and Bown 1997a, b). Considering the occurrence of dimorphism and polymorphism in heterococcolith morphology in some living coccolithophores, this approach may lead to an overestimation in terms of the number of fossil species (Jordan and Chamberlain 1997). However, if the morphological groups of *Calcidiscus* and other common coccolithophores, such as *E. huxleyi* are in fact separate species, then minor differences in the morphology of fossil coccoliths classified as a single taxa may reflect the presence of additional extinct biological groups.

Speciation and evolution

Geisen et al. (2002) believe that the different groups of *Calcidiscus* are an example of cryptic speciation within coccolithophores. Such a phenomenon has been reported within the extant planktic foraminifera *Orbulina universa* by de Vargas et al. (1999). Three genetically distinct populations with different distributions were recorded from the analysis of 18S rDNA. Subsequent detailed morphological observations of the three genotypes of extant *O. universa* indicated that they are distinguishable on the basis of pore structure and test thickness (de Vargas et al. 1999). A similar situation was revealed within the species *Globigerinella siphonifera* by Huber et al. (1997) and indicates that separate genetic groups of planktic foraminifera can be almost indiscernible based on the morphology of their skeletons. In the case of *Calcidiscus*, significant morphological differences have been demonstrated between the hetero- and holococcolith morphology of at least two of the three living groups. These differences in heterococcolith diameter and the number of elements, the nature of the central-area and the gross morphology of the holococcolith-bearing stage suggest that *Calcidiscus* does not display cryptic speciation to the same extent as reported in some groups of planktic foraminifera.

In terms of the various hypotheses proposed for speciation in pelagic organisms (see Norris 2000 for a review), there are three ways of interpreting this evolution. Given the overlap in geographical ranges, which has been demonstrated between *C. leptoporus* and *C. quadriperforatus*, the evolution of these two lineages may have taken place in sympatry. The different ecological preferences and strategies of these two groups in the plankton (Renaud et al. 2002) suggest that a possible mechanism for such sympatric evolution may be ecological specialization. However, if the dominance of these two groups at different times of the year at Bermuda (Renaud and Klaas 2001) reflects differences in the timing of their sexual reproduction, then it may be possible to invoke seasonal parapatry as the mechanism for this evolution (Norris 2000). On the other hand, whilst the different groups of *Calcidiscus* display overlapping geographic and bathymetric ranges, Beaufort and Heussner (2001) have suggested that large and small *Calcidiscus* cells grow preferentially at different depths in the water column. This would agree with a depth parapatric mode of speciation, where speciation occurs by a change in the depth of reproduction (Pierrot-Bults and Van der Spoel 1979).

Knappertsbusch (1990, 2000) and Knappertsbusch et al. (1997) have described numerous morphological groups of *Calcidiscus* in the geological record based on the diameter and number of elements of the distal shield of isolated coccoliths. The appearance and disappearance of these forms during the last ca. 20 Ma has been interpreted in terms of a complex pattern of microevolution in *Calcidiscus*, including cladogenesis, phyletic divergence and stasis. However, Knappertsbusch (2000) commented that these morphological changes could also represent ecophenotypic variation in response to changing paleoceanographic conditions. On the basis of the evidence resulting from the CODENET project, we can now conclude that most of the morphological variation in extant *Calcidiscus* is genetic in origin. If the morphologic groups of *Calcidiscus* defined by Knappertsbusch (1990, 2000) and Knappertsbusch et al. (1997) in geologic sediments also reflect the presence of separate biological groups, then widespread speciation has taken place during the history of this coccolithophore.

Sáez et al. (2003) have calculated the divergence of *C. leptoporus* and *C. quadriperforatus* as ca. 12 Ma using the molecular clock calibration technique. Comparison between this date and the phylogenetic tree of Knappertsbusch (2000) is complicated by the fact that his three extant groups or morphotypes of *Calcidiscus* and their occurrence in the fossil record are defined exclusively on the diameter and number of elements of the distal shield. The morphological analysis of *Calcidiscus* coccoliths presented here has revealed that size alone may be insufficient to define some intra-specific groups of this coccolithophore, and other features such as the nature of the central-area on the distal shield of coccoliths were found to be significant. Nevertheless, in the tree of Knappertsbusch (2000), several large-sized fossil morphotypes, including his extant morphotype L evolve between 10 and 11 Ma. This period falls within the limits of the molecular clock date (9.96–13.18 Ma), which suggests that the event that Sáez et al. (2003) have dated may be visible in the fossil record of *Calcidiscus*. However, a reassessment of *Calcidiscus* morphology during this time period is required, taking into account

the nature of the central-area of coccoliths as well as their diameter and number of elements.

Outlook

The GEM (Global *Emiliania* Modeling initiative) and EHUX (Coccolithophorid dynamics: the European *Emiliania huxleyi* programme) projects demonstrated that multidisciplinary work focusing on a single species of coccolithophore, namely *E. huxleyi*, holds great potential for the understanding of this plankton group as a whole. The subsequent CODENET research project sought to apply this philosophy to six key taxa: *Gephyrocapsa* spp., *Calcidiscus leptoporus*, *Coccolithus pelagicus*, *Umbilicosphaera sibogae*, *Helicosphaera carteri* and *Syracosphaera pulchra*. This review indicates that living *Calcidiscus* also holds great potential as a test organism to develop theories regarding the biodiversity and evolution of coccolithophores. The picture we have presented is by no means complete but is a first attempt at investigating this important coccolithophore using a multidisciplinary approach. Many existing questions still need to be answered and several new ones have arisen which will hopefully stimulate further research into fossil and living *Calcidiscus*.

An obvious task is the isolation and maintenance of *C. leptoporus* S in laboratory culture. This will provide material for ecological and physiological tests as well as molecular genetic analysis to confirm whether this group is in fact separate from the other two. With all three groups of *Calcidiscus* in culture the possibility exists to conduct immunological analysis to characterize their polysaccharides in the manner that Young and Westbroek (1991) have for *E. huxleyi*. Given that the speculation of different life cycles in the three groups of *Calcidiscus* is based on the discovery of a single combination coccosphere (Geisen et al. 2000), observation of phase changes in both *C. quadriperforatus* and *C. leptoporus* S is an important goal.

Whilst culture work is the main priority for further research into *Calcidiscus*, additional plankton observations, in particular from time series stations, will help to define more clearly the ecological preferences and bathymetric, temporal and geographic ranges of the three groups within this coccolithophore. In addition, potential exists for the reassessment of the patterns in fossil *Calcidiscus* coccolith morphology given the importance of additional morphological criteria presented here. In particular, stratigraphic confirmation of the evolutionary events indicated by molecular genetics is an important goal.

Finally, the value of the approach to the investigation of a single coccolithophore presented in this review will hopefully stimulate similar research into other common taxa.

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Species level variation in coccolithophores

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Summary

Coccolithophores are an ideal test group for investigating fine-scale differentiation within the phytoplankton since their taxonomy is rather well-documented and their biomineralised periplasts – the coccoliths – provide a rich suite of qualitative and quantitative morphological characters and a uniquely extensive fossil record. In addition, extant coccolithophore species can be grown in culture and hence are available for studies of morphological variability under controlled conditions, molecular genetic studies and cytological research.

For the CODENET project the following extant species with seemingly global occurrence and spanning the biodiversity of coccolithophores were selected: *Coccolithus pelagicus*, *Calcidiscus leptoporus*, *Umbilicosphaera sibogae*, *Syracosphaera pulchra*, *Gephyrocapsa oceanica*, and *Helicosphaera carteri*. These were used as case studies to elucidate their species level biodiversity. The key merit of our approach was the use of multiple, independent lines of evidence, so as to remove the ambiguity implicit in any one type of study. In each case morphotypes of previously uncertain significance have been shown to represent discrete genotypes and probably well-separated species, with divergence times ranging

from about 100 kyrs to >10 myrs. The evolutionary significance of these results is discussed.

Introduction

Oceans cover roughly seventy percent of the earth's surface. With few exceptions, minute photosynthetic primary producers such as diatoms, dinoflagellates, silicoflagellates and coccolithophores inhabit the euphotic zone of this vast expanse. This phytoplankton forms the basis of the marine food chain and plays an important role in geochemical cycles. Knowledge of species level biodiversity and speciation is thus important for understanding marine ecology and biogeochemistry. Moreover, for geologists seeking to maximize biostratigraphic and paleo-oceanographic data retrieval from fossil assemblages, reliable fine scale taxonomy is critical.

Coccolithophores are a group of marine calcifying algae belonging to the division Haptophyta (Jordan and Chamberlain 1997). The conventional interpretation of coccolithophore systematics is that there are about 120 well-described heterococcolithophore species (Jordan and Green 1994), with, in almost all cases, inter-oceanic distributions within broad ecological boundaries (Jordan and Kleijne 1994; Young and Bown 1994; Jordan and Chamberlain 1997). A perception of very widely distributed, rather homogeneous species is well supported by geological evidence of synchronous, apparently sympatric evolution across the world oceans and for near synchronous (on scales of less than a few thousand years) extinction events (Chepstow-Lusty et al. 1992; Wei 1993; Wei and Shilan 1996). Research over the past decade via the integration of data from various sources (morphology, life-cycles, and molecular studies) has greatly refined our knowledge of fine scale diversity in this group. This has led to the development of hypotheses of causation in terms of ecophenotypic or genotypic variation. New data have come from different sources, which will subsequently be discussed briefly.

Morphology

The primary source of data has been morphometric investigation of selected taxa using plankton, sediment trap, culture, surface sediment samples, and often time series studies of geological samples. On the basis of quantifiable morphometric parameters – mostly coccolith size, but also the number of elements (in *Calcidiscus* spp.) and the bridge angle (in *Gephyrocapsa* spp.) – this has led to the identification of sub-morphotypes and morphological gradients within species (Bollmann 1997; Knappertsbusch et al. 1997; Knappertsbusch 2000).

In addition to quantitative methods, qualitative morphological data including shield structure, crystallographic axis orientation (Young et al. 1992; Young et al. 1999), suture lines, and ornamentation of coccoliths (Kleijne 1993) have provided an important additional source of information about intra-specific variation. Ex-

amples include *Calcidiscus* spp., where the expression of suture lines and the appearance of the zone surrounding the central pore have proved to be crucial for the understanding of the diversity within the genus (Kleijne 1993). In *Helicosphaera* spp., the stability of fine morphological characters in the central area was validated as a species discriminating feature (Geisen 2003). In *Umbilicosphaera* spp., mono- versus bicyclic shield structure and central area features were used to differentiate species (Geisen 2003).

Ecological / biogeographical separation

As the concept of biological species requires reproductive isolation, a sound knowledge of the geographical and ecological ranges of the putative morphotypes is important. Evidence here stems both from the biogeographical mapping of distributions of differing morphologies and from studies of species associations from sediment trap time series. Different factors have been identified: In *Coccolithus* spp., there appears to be a spatial variation, separating a subarctic species from a temperate species with partially overlapping ranges (Cachao and Moita 2000); in *Calcidiscus* spp., Renaud and Klaas (2001) have demonstrated a temporal succession of morphologies; and finally the two "pseudo-cryptic" species ("pseudo-cryptic" speciation is speciation taking place without obvious change in morphology – for a review refer to Knowlton 1993 and references therein) detected in *Syracosphaera* apparently share the same habitat (Geisen et al. 2002).

Culture studies

A critical test for the interpretation of fine morphological differences is whether these features are stable in culture. At the most basic level if two monoclonal cultures consistently show different morphologies when grown under similar conditions then we can conclude that this morphological difference is genotypic in origin. Further testing comes from studies of the degree of morphological variation within monoclonal strains grown under varying ecological conditions. Doing this enables assessment of the degree of ecophenotypic variation, which can occur within a single genotype.

This method was first applied to coccolithophores by Young and Westbroek (1991) to assess the different effects of genotypic and ecophenotypic variation in *Emiliania huxleyi*. However, isolating and maintaining species in clonal cultures is labor intensive and since then further tests have only been performed on *Calcidiscus leptoporus* (Quinn et al. 2003). The CODENET keystone species and a number of additional species isolated during the project have now provided the opportunity for a more detailed study of the morphological variability of coccolithophores in culture. Data here include quantitative and qualitative morphological characters as described above (Geisen 2003).

Genetic separation

A set of molecular markers was used to study the extent of genotypic variation between and within recognized morphological species. Different genes from the nucleus and the chloroplast – with varying rates of nucleotide substitution – were sequenced. The slowly evolving nuclear gene 18S rDNA was used to quantify inter-specific differences, reconstruct the molecular phylogeny of coccolithophores and other Haptophyta, and infer times of divergence in these groups (e.g. Medlin et al. 1997; Edvardsen et al. 2000; Fujiwara et al. 2001). Within the CODENET project, we used it for the same purposes, extending our analyses to a much larger set of species (Sáez et al. 2003; Sáez et al. this volume). Faster evolving plastid genes have also been used for resolving coccolithophorid and Haptophyta phylogenies: *rbcL* (Fujiwara et al. 2001) and *tufA* (Sáez et al. 2003).

In addition to the nuclear 18S rDNA, including the very fast-evolving ribosomal internal transcribed spacer region (ITS rDNA), the molecular marker *tufA* was also sequenced in isolates of the same species, to assess the biological significance of phenotypic variation observed within recognized morphological species (Sáez et al. 2003; de Vargas et al. and Quinn et al. this volume.).

Although DNA has been amplified from single cells using the Polymerase Chain Reaction (PCR) technique (e.g. de Vargas et al. 1999), this has not yet been successfully applied to coccolithophores. So, our molecular work still depends on cultures to produce sufficient cell biomass for DNA amplification. This limits the number of analyses which can be made, but conversely the use of cultures means that detailed morphological analyses can be directly correlated with molecular results. As the number of strains in culture was a limiting factor, our approach was to sample genes from different loci – the chloroplast and the nucleus – to obtain, if possible, concordant results.

Molecular genetic data can be used not only to test whether putative fine-scale morphological variation is genotypic in origin but also to provide estimates of the likely time of divergence. Our data indicate that both 18S rDNA and *tufA* of coccolithophores evolved in a clock-like manner and basic calibration of this record is possible (Sáez et al. 2003). There are substantial uncertainties in any molecular clock estimate, nonetheless, it is possible to use genetic distance data to infer order of magnitude estimates of divergence times and hence to discriminate between possibilities such as that pairs of genotypes diverged within the last 1 Ma or several Ma ago.

Life-cycles and holococcolith phase differentiation

A fifth source of data has come from recognition of alternate life-cycle phases. It is now clear that the typical life-cycle of coccolithophores consists of independent haploid and diploid phases, both of which are capable of indefinite asexual reproduction (Billard and Inouye this volume). Both phases usually produce coccoliths but via distinctly different biomineralisation processes resulting in consistent structural differences. In the diploid phase biomineralisation occurs intra-cellu-

larly and produces heterococcoliths formed of radial arrays of complex crystal-units. In the haploid phase biomineralisation occurs extracellularly and produces holococcoliths formed of numerous minute euhedral crystallites. Evidence of this stems from culture observations of phase transitions (e.g. Parke and Adams 1960), observations of meiosis and syngamy (Gayral and Fresnel 1983), chromosome counts (Rayns 1962; Fresnel 1994), flow cytometry on cultured clones (Probert unpubl.), and observations of combination coccospheres from natural populations (for a review refer to Cros et al. 2000; Cros and Fortuño 2002; Geisen et al. 2002).

Thus the two phases potentially provide independent morphological evidence of differentiation. This potential has been realized in several cases, particularly through the recognition of rare combination coccospheres produced during phase transitions but also by the direct observation of phase changes in culture.

Integrative interpretation

Combining data from the sources mentioned above has allowed us to investigate fine-scale genotypic variation within the selected coccolithophores. Although we have not been able to gather evidence from all the various methods for all the species studied, there are strong patterns emerging, which allows for some generalization even if some information is missing. The different strands of evidence have produced significant evidence of different levels of genotypic variation within conventional species, which we will discuss in this review.

Results

In this section we present the data from each of the six CODENET species. Each case study was different but some strong patterns emerged. Although this is mainly a review, some previously unpublished data from our own observations are included as well. The sequence in which we present the various sources of data roughly represents the sequence in which the evidence became available and in some cases – as in *Calcidiscus* and *Coccolithus* – demonstrates how the introduction of further available data can radically change the interpretation.

***Umbilicosphaera sibogae* and *U. foliosa* (Plate 1)**

Two varieties (*U. sibogae* var. *sibogae* and *U. sibogae* var. *foliosa*) have traditionally been recognized in this species based largely on coccosphere characters. There are correlative differences in coccolith morphology but it had been speculated that these were a consequence of the different coccosphere morphology. Hence alternative hypotheses were that these varieties were different life-cycle stages of a single species or discrete taxa. *Umbilicosphaera* was selected as a CODENET species to conduct a case study on infra-specific variation of two

closely related taxa with an opportunity to study the evolutionary history as well. Based on molecular differences Sáez et al. (2003) have raised the two varieties to species rank, *U. sibogae* and *U. foliosa*, and this taxonomy is followed here.

Quantitative and qualitative morphology

The coccolithophorid genus *Umbilicosphaera* comprises four extant species (Winter and Siesser 1994) and two well-established extinct fossil species (Young 1998). The most common extant species *U. sibogae* and *U. foliosa* show a broad inter-oceanic occurrence and are common in the fossil record but there are few reliable data on their first occurrence. The genus *Umbilicosphaera* has a continuous and well-documented fossil record back to the Early Miocene (23 Ma) (Young 1998). Although McIntyre and Bé (1967) state that the two species can easily be distinguished on morphological grounds there has been some confusion in the taxonomy as Okada and McIntyre (1977) recombined *Cycloplacolithus foliosus* into *U. sibogae* var. *foliosa* on the basis that “extremely rare specimens having both types of coccoliths on a single coccosphere were observed”. Curiously this taxonomic separation has been upheld by later researchers, even though research on *Umbilicosphaera* demonstrated a stable morphology under culture conditions and diagnostic cytological differences between the species (Inouye and Pienaar 1984). Our own research – with data from cultures, natural samples, and sediments – has now validated these findings and it seems likely that Okada and McIntyre (1977) distinguished the coccoliths of the two varieties using the expression of the suture lines, which is one of the least reliable criteria for species determination (see below and Young et al. this volume).

The two species exhibit significant diagnostic differences in both coccolith and coccosphere morphology. *U. foliosa* forms a compact spherical coccosphere consisting of up to 25 interlocking placoliths (Figs. 1, 2) which are circular with a narrow central opening typically with a few hook-like spines protruding into the central opening (Figs. 2, 7). Both shields are bicyclic and convex distally, and the proximal shield is smaller than the distal shield (Figs. 8, 10). The proximal shield is composed of R-units and the distal shield of V-units. The inner half of the elements on the distal shield are imbricated dextrally and have straight sutures, the outer half kinked, with sinistral imbrication and serrated sutures (Figs. 7, 9). The central opening is spanned by an organic membrane (Figs. 1, 8). The cells are not colonial and the protoplast fills the entire coccosphere (Fig. 5). Cells are non-motile, but flagellar bases are present, uncalcified body scales are absent (Inouye and Pienaar 1984). Although intensive research has been conducted on cultures both within CODENET, and by other researchers (Inouye and Pienaar 1984), no phase changes have been observed and the life-cycle associations of the *Umbilicosphaera* spp. remain unknown.

In contrast, *U. sibogae* forms a large spherical to sub-spherical coccosphere consisting of 40 to more than 100 partly interlocking placoliths (Figs. 3, 4, 6). The placoliths are circular with a large central opening (Figs. 11, 13), the proximal shield is flat and typically larger than the convex distal shield (Fig. 12). Both shields are monocyclic, with the proximal shield composed of R-units and the

distal shield of V-units. Elements on the distal shield are imbricated dextrally, sutures straight on the inner part of the rim, then kinked and incised laevogyre on the outer part of the rim (Fig. 11). An organic membrane spans the central opening (Fig. 12). The species is semicolonial, with each coccosphere typically containing 1–2, occasionally four cells which do not fill the entire coccosphere (Fig. 6). Cells are non-motile, but flagellar bases are present (Probert unpubl.). As in *U. foliosa*, uncalcified body scales are absent (Probert unpubl.).

Working with sediment trap material, Baumann and Sprengel (2000) successfully used pore size and distal shield diameter to distinguish the two species and their data show little inter-annual size variation for *U. sibogae* coccoliths. In addition to this, own measurements of coccolith rim width and shield diameter reveal a distinct morphospace for the two taxa and no gradualistic morphological shift between them since their first occurrence in the fossil record.

Culture studies and life-cycles

Although intensive research has been conducted on cultures within CODENET, and also by other researchers (Inouye and Pienaar 1984) the life-cycle associations of the *Umbilicosphaera* spp. remain unknown. Culture studies in CODENET have, however, demonstrated the morphological stability of the two species in culture. Strains of both species have been maintained in culture for several years. During this period, the cultures were repeatedly checked by both light microscope (LM) and scanning electron microscope (SEM) but no evidence whatever of transitions between the two species was observed. A range of ecological conditions – mainly light and temperature – was tested and no significant variation in coccolith size and characters was detected.

Molecular studies

Three genes were sequenced: *rbcL* (Fujiwara et al. 2001), 18S rDNA and *tufA* (Sáez et al. 2003). All of them show a high number of substitutions between the varieties. Based on the differences in morphology and the molecular differences Sáez et al. (2003) concluded that the two variants are indeed distinct biological species. Accordingly they suggest referring to them as separate species.

Estimates from a molecular clock (*tufA* tree) date the divergence of the lineage leading to the two species at 5.6 (± 1.2) Ma (Sáez et al. 2003). Our own biostratigraphic work however dates the first occurrence of *U. sibogae* at 2.2 Ma and that of *U. foliosa* at 2.9 Ma, so it can be hypothesized here that *U. sibogae* and *U. foliosa* are not direct sister taxa or differentiated morphologically much later than they did genetically.

Status of taxa

All the data indicate that *U. sibogae* and *U. foliosa* are discrete species. This includes morphometric evidence that the coccolith morphotypes do not intergrade and the recognition of additional species based on qualitative characters. Transi-

tional morphotypes have not been observed in culture. Molecular data have revealed a significant number of substitutions between the species and point to a divergence time between 4.4 and 6.7 Ma. Nonetheless, the two taxa cluster together on all molecular trees so they are clearly closely related, as suggested by the coccolith morphology.

Plate 1 *Umbilicosphaera* spp.

Fig. 1: SEM of two *U. foliosa* coccospheres. *U. foliosa* cells are often found in clusters of up to four cells. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 69.

Fig. 2: SEM of two *U. foliosa* coccospheres. Note the presence of hook like protrusions in the central opening. Although this looks like a potential taxonomic character, cells with both types of coccoliths have frequently been observed. Water sample, N. Atlantic, R/V *Meteor* cruise 38-1, station 11.

Fig. 3, 4: SEMs of *U. sibogae* coccospheres. Note organic membrane in Fig. 4 spanning the central opening of some coccoliths. Water sample, S. Atlantic, off Namibia, R/V *Meteor* cruise M48-4, station 470 (Fig. 3) and station 20 (Fig. 4).

Fig. 5: Light micrograph of a cluster of four *U. foliosa* cells. Culture sample (ESP 6M1), western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2.

Fig. 6: Light micrograph of a dividing *U. sibogae* cell. *U. sibogae* cells can be semi-colonial with typically two cells in a single coccosphere. Note the large extracellular space. Culture material (ASM 39), western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2.

Fig. 7: SEM of the bicyclic distal shield of an *U. foliosa* coccolith. Sediment trap sample, Indian Ocean, off Somalia.

Fig. 8: SEM of the bicyclic proximal shield of *U. foliosa* coccoliths. Note the organic membrane spanning the central opening. Culture sample (ESP 6M1), western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2.

Fig. 9: SEM of the bicyclic distal shield of *U. foliosa* coccoliths. Note the straight suture lines of the inner cycle in contrast with the ragged suture lines of the outer cycle. Water sample, N. Atlantic, R/V *Meteor* cruise 38-1, station 13.

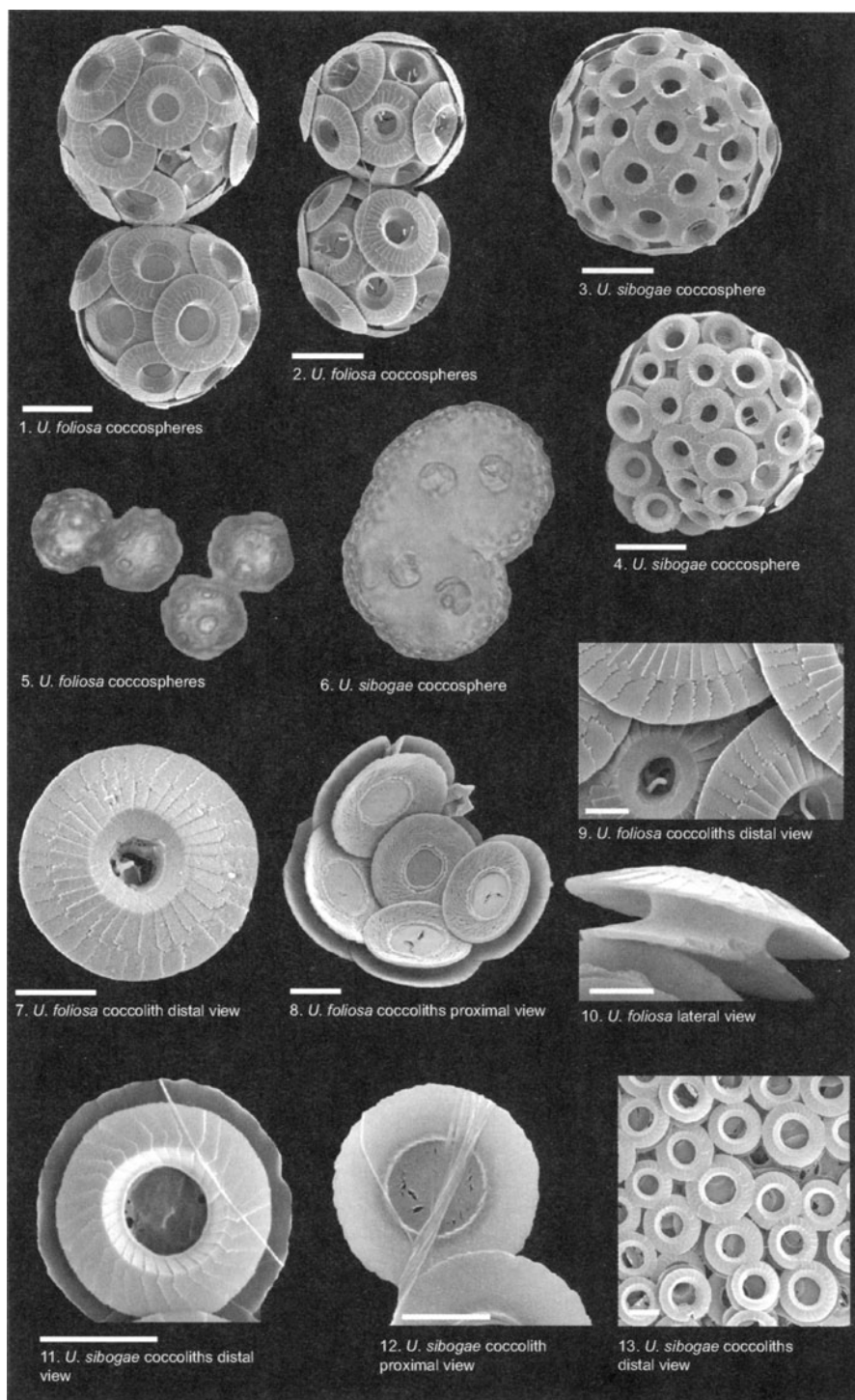
Fig. 10: SEM of an *U. foliosa* coccolith in lateral view. The distal shield is larger than the proximal shield. Water sample, western Pacific, Miyake-jima island, Ibo Port, Japan.

Fig. 11: SEM of the monocyclic distal shield of an *U. sibogae* coccolith. Proximal shield larger than distal shield. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 59.

Fig. 12: SEM of the monocyclic proximal shield of *U. sibogae* coccoliths. Note the organic membrane spanning the central opening. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 59.

Fig. 13: SEM of *U. sibogae* coccoliths from a single coccosphere. Note the size variation of the central opening and the rim. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 15.

Scale bars: Figs. 1–4: 5 μ m, Figs. 5, 6: not to scale, Figs. 7, 8, 11–13: 2 μ m, Figs. 9, 10: 1 μ m.



***Coccolithus* spp. (Plate 2)**

Coccolithus pelagicus (Wallich 1877) is one of the most robust and longest ranging of extant coccolithophores and its life-cycle is exceptionally well documented (Parke and Adams 1960; Rowson et al. 1986; Geisen et al. 2002). It appeared in the early Paleocene and it is common throughout the Cenozoic (Perch-Nielsen 1985), although this very long fossil record is based on a very broad species concept.

Today this species is commonly found in high latitudes, where it is known from the North Atlantic and the subarctic area (McIntyre and Bé 1967; Geitzenauer et al. 1977; Okada and McIntyre 1977; Samtleben and Schröder 1992). In addition, this genus is observed in low abundance in upwelling areas (e.g. Cachao and Moita 2000). Two morphotypes have been identified based on coccolith characters – a large, temperate form and a smaller, subarctic form. The morphotypes have generally been considered to represent ecophenotypic end-members of a single rather variable species. Recently however, based on life-cycle observations (Geisen et al. 2002) and genetic studies (Sáez et al. 2003), the species character of the putative ecophenotypes has been conclusively demonstrated and consequently named *C. pelagicus* (the subarctic species) and *C. braarudii* (the temperate species) and this taxonomy is followed here.

Quantitative and qualitative morphology

A range of studies with material from sediment trap, cultures and seawater samples have been carried out on the qualitative morphology of *Coccolithus pelagicus sensu lato* using both SEM and LM. Baumann et al. (2000) have used material from sediment traps from a north-south transect in the North Atlantic Ocean and demonstrated a clear change from a unimodal size distribution of the placoliths for the northern locations to a bi-modality in the more temperate locations. However, these data, based on coccolith rather than coccosphere measurements proved difficult to interpret due to the broad overlap in coccolith size between the two morphotypes. If measurements are performed on coccospheres, the two populations become clearly separable, with the temperate populations (*C. braarudii*) being dominated by large coccospheres (Fig. 1) and the subarctic populations *C. pelagicus* by small coccospheres (Fig. 5). The two morphotypes share most of their qualitative characters. However, in the large form the central area is open and spanned by a cross-bar (Figs 2, 3) whereas the central area is usually closed in the small form (Figs 6, 7). There is however evidence that these characters are secondary, size dependent characters since small specimens of the temperate form can have closed central areas and large specimens of the arctic form can have bars and open central areas.

Due to the nature of the morphological evidence there was a strong need to test whether the observed variations were ecophenotypic or genotypic in origin. Culture studies of both morphotypes under a range of different temperatures and light levels demonstrated morphological stability within each type, indicating that the

differences in morphology (coccolith and coccosphere size and shape) are under genetic control.

Life-cycles and holococcolith phase differentiation

From a synthesis of observations on holococcolith morphology in cultures and plankton samples Geisen et al. (2002) demonstrated that the temperate and sub-arctic populations of *Coccolithus* produce different holococcoliths in the alternate life-cycle phase (Figs. 4, 8 respectively). Evidence here arises from both culture studies (Parke and Adams 1960; Rowson et al. 1986; Probert unpubl.) and life-cycle associations from combination coccospheres (Samtleben and Bickert 1990; Samtleben and Schröder 1992; Winter and Siesser 1994; Baumann et al. 1997). The holococcolith types have the same rim structure. The morphology of the central area, however, is different. In the holococcolithophore stage of the temperate *Coccolithus*, the central area consists of a central ellipse of crystallites with spokes radiating towards the rim (Fig. 4). The holococcolithophore stage of the subarctic *Coccolithus* however features coccoliths with the calcite rhombohedra arranged in parallel rows with each crystal lying on one face and partly touching adjacent faces (Fig. 8).

On the basis of life-cycle studies and morphological observations Geisen et al. (2002) amended the taxonomy and recombined *Coccolithus* to include two subspecies, *C. pelagicus* ssp. *pelagicus* and *C. pelagicus* ssp. *braarudii*.

Ecological and biogeographical separation

The two extant *Coccolithus* species show different, but partly overlapping biogeographies. *C. pelagicus* prefers colder, sub-arctic water masses with temperatures ranging from -1°C–14°C (Okada and McIntyre 1979; Winter et al. 1994) and *C. braarudii* prefers temperate waters and upwelling regimes (Baumann et al. 2000; Cachao and Moita 2000; Geisen et al. 2002) with optimal growth conditions in a temperature range between 13–18°C (Cachao and Moita 2000). The discovery that extant *Coccolithus* consists of two species with significant differences in ecological preference and geographic distribution can shed a new light on the interpretation of results from the paleobiogeography of *Coccolithus* spp. (Ziveri et al. this volume).

Molecular studies

Only one sub-arctic strain was available in culture so a multi-gene approach was used to compensate for the lack of strains. The two subspecies of Geisen et al. (2002) turned out to be identical on the conservative 18S rDNA gene. However, the faster evolving genes *tufA* and ITS rDNA showed variation between the two subspecies, whereas no net variation was observed among eight strains of *C. pelagicus* ssp. *braarudii* (Sáez et al. 2003). Estimates from a molecular clock date the divergence of the two taxa at 2.2 (± 0.6) Ma (Sáez et al. 2003). On the basis of

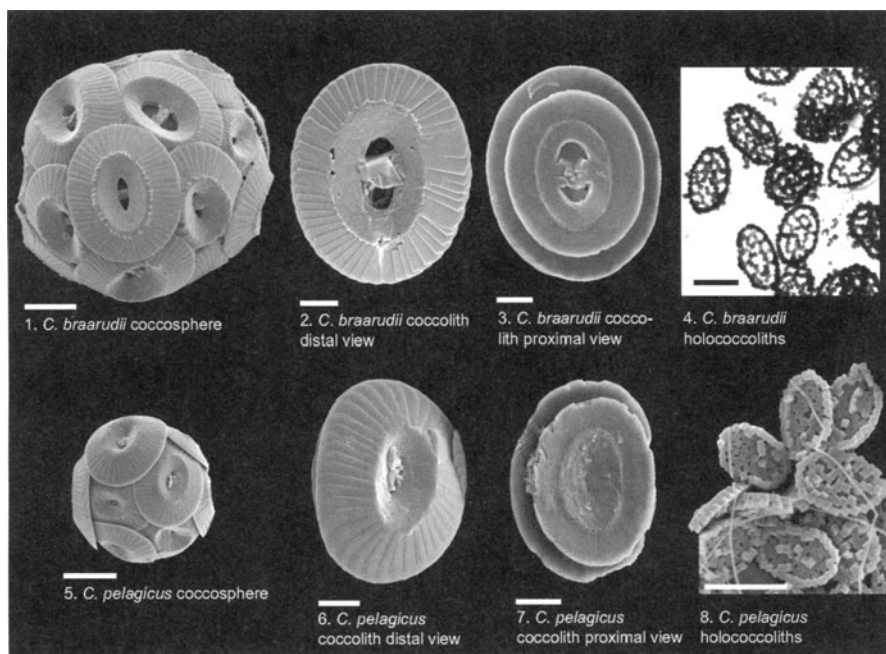


Plate 2 *Coccolithus* spp.

Fig. 1: SEM of a *C. braarudii* coccosphere. This species was previously known as the large, temperate morphotype of *C. pelagicus*. Water sample, S. Atlantic, off Namibia, R/V *Meteor* cruise M48-4, station 476.

Fig. 2: SEM of a *C. braarudii* coccolith in distal view. Culture sample (AS55T), western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2.

Fig. 3: SEM of a *C. braarudii* coccolith in proximal view. Sediment trap sample, S. Atlantic. Image courtesy Babette Böckel, Univ. Bremen.

Fig. 4: Transmission electron micrograph of the holococcolithophore stage of *C. braarudii*. This stage was previously described as “*Crystallolithus braarudii*”. Note the central ellipse of crystallites with radial spokes connected to the rim. Culture sample (LK1), SW France, off Arcachon.

Fig. 5: SEM of a *C. pelagicus* coccosphere. This species was previously known as the small, arctic morphotype of *C. pelagicus*. Water sample, N. Atlantic, off Iceland.

Fig. 6: SEM of a *C. pelagicus* coccolith in distal view. Culture sample (IBV 74), N. Atlantic, off Iceland.

Fig. 7: SEM of a *C. pelagicus* coccolith in proximal view. Water sample, N. Atlantic, off Iceland.

Fig. 8: SEM of the holococcolithophore stage of *C. pelagicus*. This stage was previously described as “*Crystallolithus hyalinus*”. Note the central area with the crystallites arranged in parallel rows. Water sample, N. Atlantic, JGOFS cruise.

Scale bars: Figs. 1, 5: 5 μm, Figs. 2–4, 6–8: 2 μm.

these important molecular divergences between the two subspecies, Sáez et al. (2003) concluded that Geisen et al. (2002) were too conservative in assigning the intra-specific subspecies rank and raised the subspecies to species rank with the large, temperate species being *C. braarudii* and the small, subarctic species being *C. pelagicus*.

Status of taxa

It has been demonstrated that this taxon includes two discrete, arctic (*Coccolithus pelagicus*) and temperate (*C. braarudii*) species (Baumann et al. 2000; Sáez et al. 2003). These can be separated according to heterococcolith size, holococcolith morphology and temperature tolerance (Cachao and Moita 2000; Geisen et al. 2002). Molecular genetic data from a range of genes support this differentiation into two discrete, but closely related species. Results from a molecular clock indicate a divergence time of the sister taxa between 1.5 and 2.7 Ma (Sáez et al. 2003).

***Helicosphaera* spp. (Plate 3)**

The extant cosmopolitan genus *Helicosphaera* is usually regarded as consisting of two species (*H. carteri* and *H. pavementum*) with three varieties in *H. carteri* (Jordan and Young 1990; Jordan and Green 1994; Jordan and Kleijne 1994). *Helicosphaera* is common in the sedimentary record and paleontologists have successfully used fine morphovariants for biostratigraphy. More than 40 species are consequently recognized in the fossil record (Perch-Nielsen 1985). The most common extant form *H. carteri* var. *carteri* has a first occurrence of Late Oligocene age and *H. carteri* var. *wallichii* of Late Miocene (Tortonian) age (Young 1998).

The three varieties of *H. carteri* – *H. carteri* var. *carteri*, *H. carteri* var. *wallichii* and *H. carteri* var. *hyalina* – were described as separate species but were considered to differ only in central area pore development and so have been recombined as varieties (Jordan and Young 1990) and have been suggested to be intergradational morphotypes (Nishida 1979). Recent evidence from molecular studies (Sáez et al. 2003) has led to the conclusion that the variants *H. carteri* var. *carteri* and *H. carteri* var. *hyalina* are separate biological species, resulting in the species *H. carteri* and *H. hyalina* and this taxonomy is followed here. Based on life-cycle inferences and morphology we argue here that *H. carteri* var. *wallichii* should equally be restored to species rank.

Quantitative and qualitative morphology

Coccoliths of the genus *Helicosphaera* can be easily distinguished from other species due to their helical flange and their comparatively large size. Jafar and Martini (1975), Theodoridis (1984) have reviewed the genus *Helicosphaera*. The three extant varieties assigned to *Helicosphaera* share a number of morphological characters. All form ellipsoidal coccospheres with spirally arranged asymmetrical heli-

coliths. The cells are flagellate and the flagellar pole is surrounded by slightly modified coccoliths, which usually exhibit a larger wing (Figs. 1, 4, 7). Characters such as wing development and presence of tooth like protrusions on the wing are strongly variable within each variety and do not show obvious variation between the varieties. The central area characters, however, are distinctly different. In *H. carteri* the central area of the helicoliths show a bar, which separates two in-line openings (pores or slits) (Figs. 1–3). In contrast the central area of *H. hyalina* is characterized by the absence of pores or slits and shows larger blanket elements (Figs. 7–9). Finally, *H. wallichii* has a central area with a bar separating two dextrally aligned, oblique slits with kinked ends (Figs. 4–6). Measurements of coccolith size within *Helicosphaera sensu lato*, based on water column, sediment, and sediment-trap samples resulted in bi- or multimodal morphospace that were difficult to interpret and could either represent genotypic or ecophenotypic variation.

During CODENET multiple *H. carteri* (Fig. 1) cultures became available and the morphological stability of coccolith size and shape under a range of environmental conditions was demonstrated. Slit/pore size and shape were remarkably variable even within the same clonal culture, however, no specimens showed the oblique pores of *H. wallichii*. Rare coccoliths lacking pores did occur but these did not show the smaller size or large blanket elements characteristic of *H. hyalina*.

A single clone of *H. hyalina* (Fig. 7) was cultured and – in comparison with *H. carteri* – exhibited consistently smaller coccolith size in addition to the central area features described above. In addition the coccospheres are typically subspherical rather than ellipsoidal. These features remained stable in culture and proved valuable for species recognition.

We did not successfully isolate *H. wallichii*, but re-examination of a coccolith sample provided previously by I. Inouye from a *Helicosphaera* culture isolated off Japan showed that this was a strain of *H. wallichii*, with all coccoliths showing oblique slits in the central area (Fig. 4). We note that his strain was used by Fujiwara et al. (2001) in their analysis of haptophyte phylogeny using *rbcL*, and named there as *H. carteri*.

A review of our large collection of scanning electron micrographs of coccoliths and coccospheres of *Helicosphaera* from all oceans revealed that on coccospheres of *H. wallichii* and *H. hyalina* all visible coccoliths showed the typical features of the species. By contrast a rather high morphological variability of the central area characters is often seen on *H. carteri* coccospheres, as shown by (Nishida 1979). Pore size is very variable, the two pores may merge into a single slit, and occasionally one or even both pores are absent. *H. carteri* coccoliths without pores are nonetheless readily separable from *H. hyalina* coccoliths by their larger size and smaller blanket elements. The findings on the natural samples hence support our morphological observations on the cultured clones. These results from morphological work indicate that there is a strong genotypic control on the morphological variation in *Helicosphaera*.

Life-cycles and holococcolith phase differentiation

Recent evidence from combination coccospheres of *H. carteri* with a holococcolithophore "*Syracolithus catilliferus*" (Figs. 10–12) (Cros et al. 2000) and combination coccospheres of the holococcolithophores "*Syracolithus catilliferus*" and "*Syracolithus confusus*" (Fig. 13) (Geisen et al. 2002) suggests that both holococcolithophores can occur on the haploid phase of the life-cycle of *H. carteri*. Cros et al. (2000) and Geisen et al. (2002) note the close morphological relationship between the two holococcoliths involved (Figs 10, 12) and explain this similarity by non-genotypic variations in the degree of calcification. Although the available examples are few and based on observations on natural populations only, they provide convincing evidence to treat both holococcolithophores as junior (heterotypic) synonyms of *H. carteri*.

We have further tentative evidence from a single possible combination coccosphere for a life-cycle association of *H. wallichii* with a holococcolithophore *Syracolithus dalmaticus* (Figs. 14–16). This is not a particularly clear example of a combination cell and could indeed be an accidental association. However, in our field samples of the variant *wallichii* the holococcolithophore *S. dalmaticus* usually occurs as well. In particular we found several specimens of both *H. wallichii* and *S. dalmaticus* in samples collected from around the island Miyake-jima, Japan in 2001. This is the location from which I. Inouye's culture of *H. wallichii* was previously isolated. So we tentatively conclude that there is evidence for differentiation of both holococcolith and heterococcolith between *H. wallichii* and *H. carteri*.

NB *Helicosphaera wallichii* (Lohmann 1902) Boudreaux and Hay (1969) has priority over *Syracolithus dalmaticus* (Kamptner 1927) Loeblich and Tappan (1966), hence – if the combination is proved – the correct name for the species will be *H. wallichii*. There is no evidence available on the haploid phase of *H. hyalina*, although it is interesting to note that the holococcoliths within the genus *Helicosphaera* have a highly distinctive ultrastructure, formed predominantly of aligned rhombohedral crystallites (Fig. 11). One further holococcolithophore, *S. ponticuliferus*, is known to have this ultrastructure and so is a prime candidate for a possible *H. hyalina* holococcolithophore.

Molecular studies

Two genes – the conservative 18S rDNA and the faster evolving *tufA* – were targeted for the two varieties *carteri* and *hyalina* within *Helicosphaera* available in culture. However, despite numerous attempts with *H. hyalina*, it was only possible to sequence 18S rDNA for *H. carteri*. The *tufA* gene was successfully sequenced from both taxa and exhibited a large number of substitutions between them. A molecular clock analysis of this variation allowed inference of a most recent common ancestor for these two taxa at 10.2±2 Ma (Sáez et al. 2003). Based on the differences in morphology and the molecular data Sáez et al. (2003) recommended that the varieties should be restored to species rank, and so named *H. carteri* and *H. hyalina*. Due to the lack of new isolates of *H. wallichii* comparative molecular data are not available for it.

Plate 3 *Helicosphaera* spp.

Fig. 1: SEM of an *H. carteri* coccosphere. The helicoliths show the typical spiral arrangement and the circumflagellar coccoliths possess enlarged flanges. The central area of this specimen shows the typical morphology with two aligned slits separated by a bar. Morphotypes with small round pores and intermediate central area morphologies have also been observed. Note the small triangular protrusions on the flange. Water sample, N. Atlantic, Portuguese shelf, R/V *Andromeda* cruise CODENET 2, station 6.

Fig. 2: SEM of a *H. carteri* coccolith in proximal view. Sediment trap sample, S. Atlantic. Image courtesy Babette Böckel, Univ. Bremen.

Fig. 3: SEM of a *H. carteri* coccolith in distal view. Sediment trap sample, S. Atlantic. Image courtesy Babette Böckel, Univ. Bremen.

Fig. 4: SEM of a *H. wallichii* coccosphere. The central area shows the typical morphology with two angled slits with kinked ends, separated by a bar. This fine morphological feature is stable in culture. Note the small triangular protrusions on the flange. Water sample, western Pacific, Miyake-jima island, Miike Port, Japan.

Fig. 5: SEM of a *H. wallichii* coccolith in proximal view. Note the kinked ends of the aligned slits. Water sample, western Pacific, Miyake-jima island, Miike Port, Japan.

Fig. 6: SEM of a *H. wallichii* coccolith in distal view. Sediment trap sample, Indian Ocean, off Somalia.

Fig. 7: SEM of a *H. hyalina* coccosphere. The central area is filled with tangentially arranged needle shaped elements. Note the small triangular protrusions on the flange. Culture sample (NAP 11), Mediterranean, off Naples, Italy.

Fig. 8, 9: SEM of a *H. hyalina* coccolith in proximal view (Fig. 8) and distal view (Fig. 9). Culture sample (NAP 11), Mediterranean, off Naples, Italy.

Fig. 10: SEM of a *H. carteri* coccosphere in the holococcolith bearing stage. This stage was previously described as “*Syracolithus catilliferus*” and is referred to as *H. carteri* HO solid-type. Water sample, N. Atlantic, off Canary Islands, R/V *Poseidon* cruise P233B, station 3.

Fig. 11: SEM of *H. carteri* coccoliths in the holococcolith stage. Water sample, Antarctic Ocean, cruise JR 48.

Fig. 12: SEM of a *H. carteri* coccosphere in the holococcolith bearing stage. This stage was previously described as “*Syracolithus confusus*” and is referred to as *H. carteri* HO perforate-type. Water sample, western Mediterranean, off Barcelona.

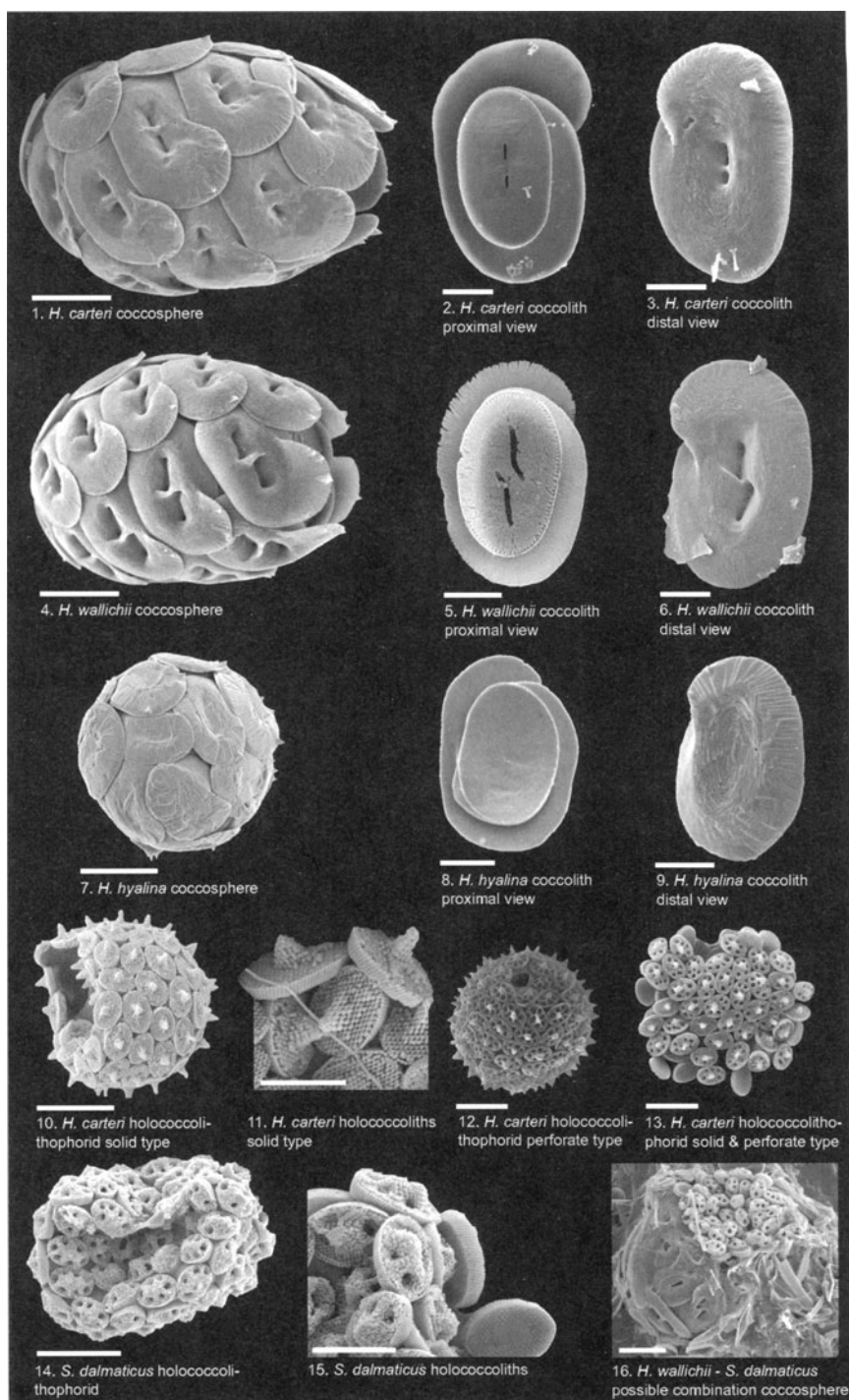
Fig. 13: SEM of a *H. carteri* coccosphere in the holococcolith bearing stage. Note that presence of coccoliths of both *H. carteri* HO solid and perforate. This is seen as an example of intraspecific variation in the degree of calcification. Water sample, NW Mediterranean, cruise MESO-96, station F2.

Fig. 14: SEM of a *S. dalmaticus* holococcolithophore. Water sample, western Pacific, Miyake-jima island, Ibo Port, Japan.

Fig. 15: SEM of a detail of *S. dalmaticus* holococcoliths. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 69.

Fig. 16: SEM of a tentative *H. wallichii* – *S. dalmaticus* combination coccosphere. Water sample, Gulf of Mexico, R/V *Gyre* cruise 92-G-03, station 9.

Scale bars: Figs. 1, 4, 7, 10, 12–14: 5 μ m, Figs. 2, 3, 5, 6, 8, 9, 11, 15: 2 μ m.



Status of taxa

Morphometric analyses again produced ambiguous patterns, which were difficult to interpret. Our key result came from culture isolation of a disputed morphovariant – *H. hyalina*. Contrary to expectations the coccolith morphology has proved stable in culture indicating again that a subtle morphological variant is under genotypic control. On the basis of our new results from morphological and molecular studies Sáez et al. (2003) have concluded that the two varieties are in fact separate, albeit closely related species and that their most recent common ancestor would have lived between 8.2 and 12.2 Ma. Although molecular data are still pending, evidence from the morphology, life-cycle data and previous culture observations strongly support that the previously described variant *H. carteri* var. *wallichii* should be considered a discrete species as well. These findings have led to the suggestion that in this species sympatric evolution of a phenotypically plastic population might lead to gradualistic change in the range of morphological variation within a single species.

***Calcidiscus* spp. (Plate 4)**

Together with the *Gephyrocapsa-Emiliania* plexus, *Calcidiscus* is probably the best documented coccolithophorid genus (Brand 1981; Knappertsbusch et al. 1997, Knappertsbusch 2000; Baumann and Sprengel 2000; Renaud and Klaas 2001; Renaud et al. 2002; Quinn et al., 2003). Like the aforementioned genera it has a broad, inter-oceanic occurrence spanning a range of ecological variation and a very good, continuous fossil record through the last 23 Ma. Three extant morphotypes have been tentatively identified, based largely on size of the coccoliths and coccospheres and there has been much speculation as to whether this variation represents distinct species or ecophenotypes. Recently, however, Geisen et al. (2002) and Sáez et al. (2003) have conclusively demonstrated that the large and intermediate morphotypes do represent distinct biological species – *C. quadriperforatus* and *C. leptoporus* respectively – and this taxonomy is followed here. A detailed review of this species complex is presented by Quinn et al. (this volume) and for reasons of completeness we summarize the available data here.

Quantitative and qualitative morphology

Three morphotypes (large, intermediate and small) were identified within extant populations of *Calcidiscus*, based on the size of coccoliths and coccospheres (for references see Quinn et al. this volume) (Figs. 1–3). The morphospace based on coccolith size measurements and element counts reveals a trimodal distribution, albeit with broadly overlapping margins and the modes compare well with the size range reported for Holocene material. It has, however, been pointed out that size measurements as a sole character are not sufficient to distinguish the morphotypes. If other, qualitative characters are added, the morphotypes become easily separable (Kleijne 1993; Baumann and Sprengel 2000; Geisen et al. 2002) (Figs. 4–9).

Research during CODENET has additionally shown that at least two of the morphotypes exhibit a stable morphology in culture under varying environmental conditions (Quinn et al. 2003; Quinn et al. this volume).

Life-cycles and holococcolith phase differentiation

Observations on plankton samples and on cultured clones of *Calcidiscus* have demonstrated that the large and intermediate morphotype independently form life-cycle associations with distinctly different holococcolithophores (Figs. 10–12). In combination with the morphological observations this led Geisen et al. (2002) to amend the taxonomy, assigning subspecies rank to the morphotypes.

Molecular studies

The two subspecies identified by Geisen et al. (2002) can be distinguished using both the conservative 18S rDNA and the faster evolving *tufA* genes. With this information Sáez et al. (2003) concluded that Geisen et al. (2002) were too conservative in using the intra-specific rank subspecies and have therefore raised the subspecies to species level. Furthermore they present evidence for two distinct genotypes within *C. quadriperforatus* and assume that this represents a case of recent cryptic speciation. In the same study they calculate the divergence time for *C. leptoporus* (the intermediate “morphotype”) and *C. quadriperforatus* (the large “morphotype”) at 11.6 ± 1.6 Ma, which correlates well with the results of Knappertsbusch (2000) from the morphological classification of *Calcidiscus* in the fossil record.

Status of taxa

Initial results from oceanographic and culture studies produced conflicting data. However, subsequent data from holococcolith morphology (Geisen et al. 2002) and a combined morphological and molecular genetic study of a large collection of culture isolates proved that the variation was predominantly genotypic (Sáez et al. 2003). This has provided further insights with respect to the occurrence and ecological preferences of the former morphotypes (Baumann and Sprengel 2000; Renaud and Klaas 2001; Renaud et al. 2003). The molecular clock and findings from the fossil record point to a relatively deep divergence of the *Calcidiscus* species. An additional surprising result from the molecular studies was the discovery of recent cryptic speciation within *C. quadriperforatus*.

***Gephyrocapsa oceanica* and related species (Plate 5)**

The genus *Gephyrocapsa* constitutes a late Neogene group of heterococcolithophores that first developed in the late Pliocene, about 3.5 Ma ago (*Reticulofenestra pseudoumbilicus* Zone, NN 15), became dominant in the Early Pleistocene, and

Plate 4 *Calcidiscus* spp.

Fig. 1: SEM of a *C. quadriperforatus* coccosphere. This species was previously known as the large morphotype of *C. leptoporus*. Water sample, western Pacific, Miyake-jima island, Miike Port, Japan.

Fig. 2: SEM of a *C. leptoporus* coccosphere. This species was previously known as the intermediate morphotype of *C. leptoporus*. Culture sample (NS 10-2), S. Atlantic, off South Africa, R/V *Agulhas* cruise MARE 2.

Fig. 3: SEM of a *Calcidiscus* sp. SMALL coccosphere. Note the kinked suture lines that can be traced into the central pore. This species was previously known as the small morphotype of *C. leptoporus*. As no holococcolithophore stage has been identified an informal classification is used. Water sample, S. Atlantic, off Namibia, R/V *Meteor* cruise M48-4, station 472.

Fig. 4: SEM of a *C. quadriperforatus* coccolith in distal view. Note the curved suture lines and the obscured zone around the central pore. Culture sample (ASM 27), western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2.

Fig. 5: SEM of *C. leptoporus* coccoliths in distal view. Note the curved suture lines that can be traced into the central pore. Water sample, western Pacific, Miyake-jima island, Chyotaru Port, Japan.

Fig. 6: SEM of *Calcidiscus* sp. SMALL coccolith in distal view. Note the kinked and ragged appearance of the suture lines that can be traced into the central pore. Water sample, western Pacific, Miyake-jima island, Chyotaru Port, Japan.

Fig. 7: SEM of a *C. quadriperforatus* coccolith in proximal view. Water sample, western Pacific, Miyake-jima island, Ibo Port, Japan.

Fig. 8: SEM of *C. leptoporus* coccoliths in proximal view. Water sample, western Pacific, Miyake-jima island, Chyotaru Port, Japan.

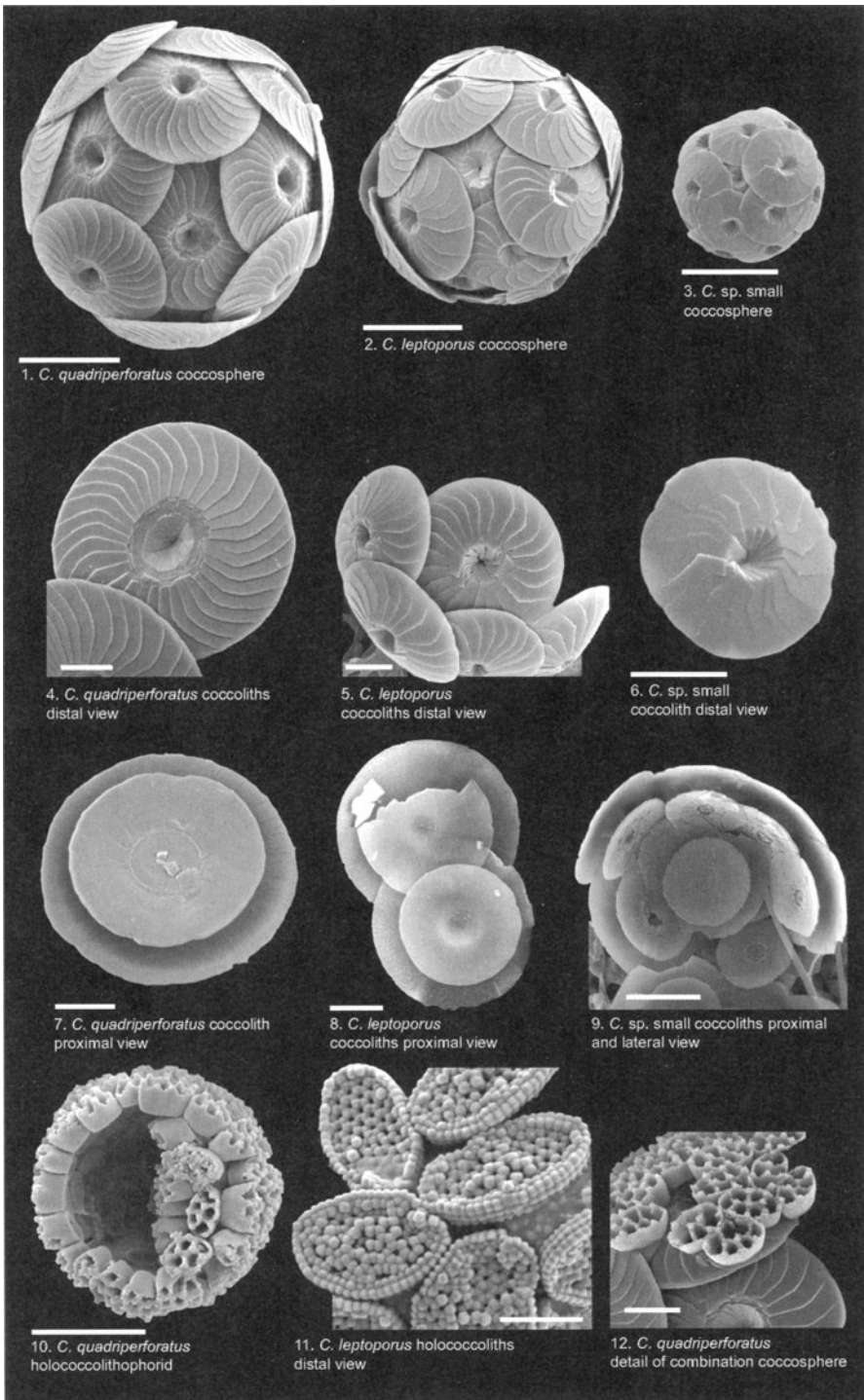
Fig. 9: SEM of *Calcidiscus* sp. SMALL coccoliths in proximal view. Water sample, N. Atlantic, R/V *Meteor* cruise 38-1, station 12.

Fig. 10: SEM of the holococcolithophore stage of *C. quadriperforatus*. This stage was previously described as “*Syracolithus quadriperforatus*”. Water sample, N. Atlantic, off the Canary Islands, R/V *Poseidon* cruise P233B, station 2.

Fig. 11: SEM of the holococcolithophore stage of *C. leptoporus*. This stage was previously described as “*Crystallolithus rigidus*”. Culture sample (NS 10-2), S. Atlantic, off South Africa, R/V *Agulhas* cruise MARE 2.

Fig. 12: Detail of a SEM of a combination coccosphere bearing coccoliths of both the holococcolithophore stage of *C. quadriperforatus* and the associated holococcolithophore stage. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 69.

Scale bars: Figs. 1–3, 10: 5 μ m, Figs. 4–9, 12: 2 μ m, Fig. 11: 1 μ m.



has yielded ecological dominance during the last 85 kyr to the descendant species *Emiliania huxleyi*. Because of the close relationship of *E. huxleyi* with genus *Gephyrocapsa*, *E. huxleyi* has been regarded as a modern ecological surrogate for the small gephyrocapsids of the Pleistocene, which it has replaced in the modern phytoplankton (e.g. Gartner 1988). Phylogenetically *E. huxleyi* is part of the *Gephyrocapsa* clade and hence needs to be considered within the group.

Gephyrocapsa is a complex genus, which shows considerable morphological variability and has been intensively studied by paleontologists in order to produce a high-resolution biostratigraphic subdivision of the Quaternary. Thus, there are many extant and extinct species classified under the genus *Gephyrocapsa*, and most of which are defined solely by morphologic characters (e.g. Pugs 1985). According to this taxonomy the modern *Gephyrocapsa* assemblage consists of four species with first appearances between 1–3.5 Ma (Samtleben 1980; Matsuoka and Okada 1990; Young 1991). However, detailed morphometry, qualitative morphological, and biogeographical work indicates that each of these species consists of different morphotypes (Bollmann 1997; Bollmann et al. 1998; Hagino and Okada 2001; Cros 2002). *Emiliania huxleyi* is the most abundant living coccolithophore and appears to have extremely broad ecological affinities, occurring in all of the main oceanic conditions. Large-scale blooms which mainly consist of *E. huxleyi*, are regularly observed during early summer in the northern North Atlantic (e.g. Brown and Yoder 1993; Holligan et al. 1993). This species is easy to maintain in culture and has been subject to intensive studies, combining observations from culture and oceanic populations (e.g. Westbroek et al. 1989; Young and Westbroek 1991; Westbroek et al. 1993; Westbroek et al. 1994; Young 1994). In addition, *Emiliania huxleyi* has a very well constrained first occurrence at only 270 ka (Thierstein et al. 1977).

Quantitative and qualitative morphology

The genus *Gephyrocapsa* was first described by Kamptner (1943) and included only one species, *G. oceanica*, which he later divided into two varieties, *G. oceanica* var. *typica* and *G. oceanica* var. *californiensis* (Kamptner 1956). Much later, the first small *Gephyrocapsa* species, *G. aperta*, was described (Kamptner 1963). Since then more than 20 species have been described within this genus on the basis of various criteria. Many of these species have been distinguished using morphologic criteria detectable with LM such as size, proportion of the central area and bridge angle (e.g. Boudreaux and Hay 1969; Hay and Beaudry 1973). Some have been cited only once, whereas others, such as *Gephyrocapsa reticulata* (Nishida 1971), lack a crossbar and, therefore, cannot be assigned to the genus *Gephyrocapsa*. Whereas large species like *G. oceanica* (Figs. 1, 2) and *G. muelerae* (Figs. 5, 6) are relatively easy to distinguish even with light microscopy it was only after the SEM replaced LM and transmission electron microscopes that some characteristic small species such as *G. ornata* (Figs. 3, 4), *G. ericsonii* (Figs. 7, 8) or *G. aperta* were accurately defined. A major step forward in the classification of *Gephyrocapsa* species came when Br  h  ret (1978) and Samtleben (1980) independently demonstrated the feasibility of distinguishing different morphologi-

cal species by using simple quantifiable morphometrical characters (mainly coccolith size and bridge angle). Comparable size criteria were applied to the entire *Gephyrocapsa* complex when Matsuoka and Okada (1989, 1990) investigated time-progressive variations in the morphology of the genus.

All extant species of the genus *Gephyrocapsa* build spherical to sub-spherical coccospheres of oval placoliths with a diagonal bridge crossing the central area (Figs. 1–8). This distinctive feature makes even the smallest specimens identifiable at generic level. Many variations of the coccolith bridge exist. Some have a high-arched bridge, whereas others have a low-profile bridge formed by fine rod-like elements.

Recently, morphological analysis of Holocene *Gephyrocapsa* assemblages revealed six dominant morphological associations (Bollmann 1997), which the author described informally, since he reserved judgment on whether these corresponded to discrete species or, in part, to ecophenotypes. The different morphotypes appear to have distinct environmental preferences with respect to temperature and productivity but do not show intermediate morphologies in intermediate ecological conditions. During CODENET only *G. oceanica* was successfully isolated and so available for study in detail in culture (C. Klaas unpubl. data). Data emerging from this work demonstrate a strong stability of morphological characters within the species.

Emiliania huxleyi (Lohmann 1902), Hay and Mohler, 1967 in Hay et al. 1967 forms spherical coccospheres consisting of fewer than 10 to up to more than 50 partially interlocking placoliths (Figs. 9–12). These oval placoliths typically are formed of T-shaped elements and have an elliptical central area. Young and Westbrook (1991) distinguished four varieties of *E. huxleyi* – types A (Figs. 9, 12), B, C (Fig. 11), and var. *corona* (Fig. 10) based on culture observations, immunochemical tests and plankton observations, although they stated that biometrical analyses do not separate these types easily. *E. huxleyi* was not a target of CODENET research but our limited culture observations have supported the pattern of stable genotypically determined morphology in culture.

Molecular studies

E. huxleyi has previously been genetically characterized in a number of studies. Medlin et al. (1996) sequenced a number of clonal strains from all oceans which were identical with regard to the chloroplastic 16S and the nuclear 18S rDNA and the spacer region between the Rubisco *rbcL* and *rbcS*. However, genetic fingerprinting techniques such as RAPDs (random amplification of polymorphic DNA) and AFLPs (amplified fragment-length polymorphism) revealed significant genetic differences between strains (for a review refer to Mueller and LaReesa Wolfenbarger 1999). Using RAPD Medlin et al. (1996) found that all strains except for one pair were genetically distinct and that this genetic diversity is reflected in the morphology and the ecological distribution of the strains. On the basis of the RAPD study by Medlin et al. (1996) the proposed variants by Young and Westbrook (1991) were formally emended.

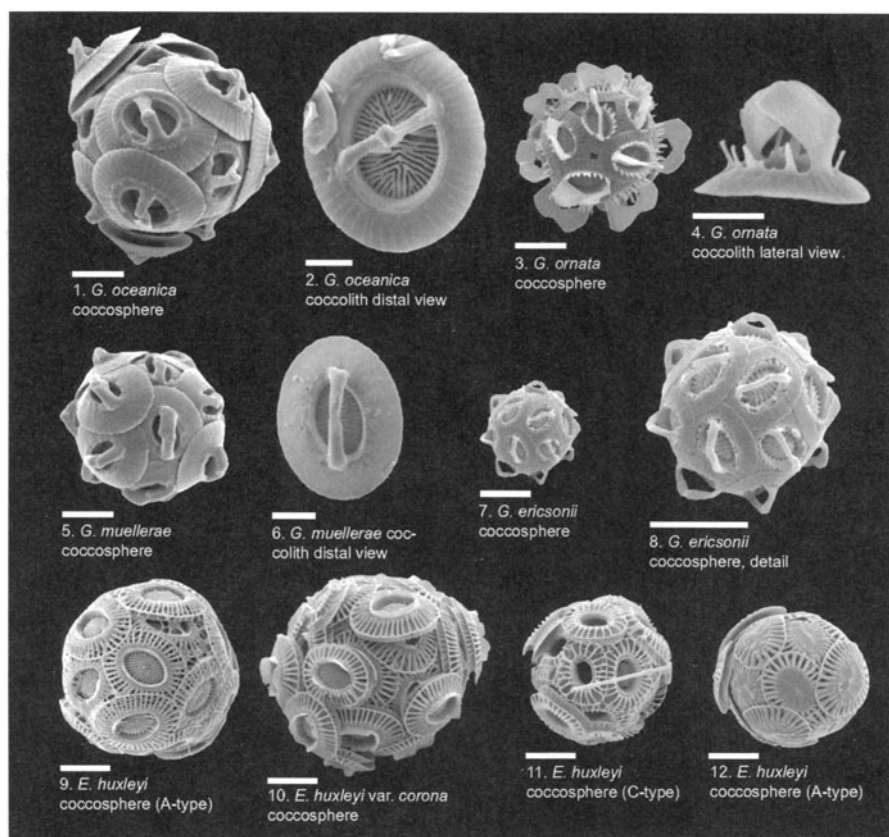


Plate 5 *Gephyrocapsa* spp. and *Emiliana* spp.

Fig. 1: SEM of a *G. oceanica* coccosphere. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 59.

Fig. 2: SEM of a *G. oceanica* coccolith in proximal view. Sediment trap sample, S. Atlantic. Image courtesy Babette Böckel, Univ. Bremen.

Fig. 3: SEM of a *G. ornata* coccosphere. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 44.

Fig. 4: SEM of a *G. ornata* coccolith in lateral view. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 69.

Fig. 5: SEM of a *G. muelleriae* coccosphere. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 69.

Fig. 6: SEM of a *G. muelleriae* coccoliths. Sediment trap sample, N. Atlantic, JGOFS cruise.

Fig. 7, 8: SEM of a *G. ericsonii* coccosphere. Fig. 16 displays an enlarged view of the same specimen. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 15.

Fig. 9: SEM of a *E. huxleyi* coccosphere. The coccoliths exhibit the A-type morphology. Water sample, N. Atlantic, R/V *Meteor* cruise 42-4B, station US 1B.

Plate 5 (cont.)

Fig. 10: SEM of a *E. huxleyi* var. *corona* coccosphere. Note the collar surrounding the central area. Water sample, N. Atlantic, off Canary Islands, R/V *Poseidon* cruise P233B, station 3.

Fig. 11: SEM of a *E. huxleyi* coccosphere. The coccoliths exhibit the C-type morphology. Water sample, N. Atlantic, R/V *Meteor* cruise 38-1, station 12.

Fig. 12: SEM of an *E. huxleyi* coccosphere. The coccoliths exhibit the A-type morphology with the central area being overcalcified. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 64.

Scale bars: Figs. 1, 3, 5, 7–12: 2 μm , Figs. 2, 4, 6: 1 μm .

Recently Iglesias-Rodríguez et al. (2002a, 2002b) further demonstrated a high degree of polymorphism in isolates of *E. huxleyi* of different geographical origin using AFLP and microsatellite loci. Gene flow and calculations of genetic diversity using population statistics are presently under way. All of these studies predict that *E. huxleyi* must be undergoing frequent sexual recombination to maintain such high diversity among populations reproducing vegetatively to maintain high biomass.

In CODENET a number of *E. huxleyi* and *G. oceanica* strains and *Emiliania huxleyi* morphotype R were sequenced (Sáez et al. unpublished). With the slowly evolving 18S rDNA all the sequences obtained are identical, which is consistent with a recent divergence of these genotypes. But isolates, either from the same morphological species or not, appear genetically distinct at the fast evolving *tufA* gene. Unlike *C. pelagicus* or *C. leptoporus*, where each morphotype was monophyletic, *E. huxleyi* and *G. oceanica* species and morphotypes were mixed in relation to the *tufA* genotypes. These unexpected findings have been interpreted to be the result of ancient (or shared) polymorphisms at the *tufA* gene, which have persisted through the speciation events in these different lineages. Thus future work regarding the genetic variability of the species in correlation to their ecological preferences remains an interesting topic and is likely to be solved using DNA fingerprinting methods, such as microsatellites or SNPs (single nucleotide polymorphism) analyses. Due to the failure to isolate other *Gephyrocapsa* and *Emiliania* species, information about their genetic variability is still lacking.

Life-cycles and holococcolith phase differentiation

The life-cycle of *E. huxleyi* consists of coccolith bearing non-motile, diploid C cells and motile, haploid, scale-bearing S cells (see Billard and Inouye this volume). Holococcoliths are not produced in the haploid stage. A third type of cells – the naked N cells – is diploid and does not appear to be part of the haplo-diplontic life-cycle, but a mutant. All cell types are capable of indefinite asexual reproduction by binary fission. Few life-cycle observations are available for *Gephyrocapsa* spp. But, as in *Emiliania* spp., the haploid stage is motile and covered with unmineralized scales (Probert unpubl. data). It is possible that the scales from S-cells of *Emiliania* and *Gephyrocapsa* may provide morphological criteria to help assess fine-scale speciation patterns but such data is not yet available.

Ecological and biogeographical separation

By measuring the reproduction rates of a large number of *E. huxleyi* and *G. oceanica* clones under the same environmental conditions, Brand (1981, 1982) demonstrated both the stability of this parameter in single clones, and considerable variation between clones. He therefore concluded that a natural population is not clonal but consists of a mixture of genotypes with different reproductive potentials (Brand 1982). However he did not claim that this differentiation could only be due to the existence of reproductive barriers: "these species (...) either undergo extensive genetic recombination with the resulting genotypes having different reproductive potentials or exist as complexes of coexisting clonal lines" (Brand 1982). Paasche (2002) points out the relative tolerance of coastal clones of *E. huxleyi* to salinity variations, contrasted by less tolerant oceanic *E. huxleyi* clones. Young and Westbroek (1991) describe *E. huxleyi* type A, B and C as well as the variant *corona* but they reserve judgment whether these are typical of distinct environments. *Emiliania* appears to have diverged into at least five discrete sub-species with partially overlapping biogeographies (Young and Westbroek 1991; Medlin et al. 1996; Findlay and Giraudeau 2001).

In the genus *Gephyrocapsa* culture studies were limited because most of the recognized species were not isolated into culture. However, the hypothesis of differential ecologies of the species has been tested in detail through derivation of a temperature transfer function based on the distribution of *Gephyrocapsa* species (Bollmann et al. 2002).

Status of taxa

In the case of *E. huxleyi* there is evidence of different levels of genotypic variation. Firstly, there is the well-documented appearance of genus *Emiliania* at about 270 ka, which has since diverged into a number of well-defined species, detectable by molecular and morphological methods. Secondly, there is evidence of a high genotypic variability at the population scale. It has been demonstrated that a population is not a single clone or genotype, but a mixture of genetically distinct clones, which are attuned to yield maximum growth rates in a range of environmental conditions. Recent molecular studies using microsatellite loci have confirmed that populations of *E. huxleyi* – separated by major oceanic boundaries – show a distinct genetical fingerprint and can thus be spatially separated.

The overall pattern of variation in *Gephyrocapsa* is comparable with *Emiliania*. A relatively young genus has diverged into a number of well-defined morphological species (*G. muelleriae*, *G. ericonii*, *G. oceanica*, *G. ornata*) most of which comprise two or more morphotypes (Bollmann 1997) which probably do represent pseudo-cryptic species. In addition, different clones of *G. oceanica* have been tested for their environmental preferences and they reveal a genotypic variability of the same nature as in *Emiliania* (Brand 1982). *Emiliania* and *Gephyrocapsa* species play an important role in geochemical cycles and knowledge of both intra- and inter-specific genotypic variation is crucial to determine which species are the most important actors in, for example, the carbon cycle. Therefore, the develop-

ment of specific markers to map the spatial and temporal variability of both *E. huxleyi* and *Gephyrocapsa* spp. remains an important target for future research.

***Syracosphaera pulchra* (Plate 6)**

Syracosphaera pulchra is the most common member of the very diverse extant genus *Syracosphaera* and the only one to have been successfully isolated into culture (Inouye and Pienaar 1988). It occurs globally in all oceans (Okada and Honjo 1973; Okada and McIntyre 1977; Winter et al. 1994) and is common in sediments. Its first occurrence in the fossil record is in the early Pliocene at 4.8 Ma. This date is based on ODP Site 664 from analysis of the NEPTUNE database (Spencer-Cervato 1999) and was confirmed in the ODP reference slide collection at the Natural History Museum, London.

As *S. pulchra* coccospheres and coccoliths are very rich in morphological characters the species was regarded as very well defined and was selected as a key species for the CODENET project, in part to act as a control species to quantify the degree of intra-specific variation. The recent discovery of “pseudo-“ cryptic species in *S. pulchra* (Geisen et al. 2002) however has shed a new light on this interpretation.

Quantitative and qualitative morphology

Coccospheres of the heterococcolith stage of *S. pulchra* are spherical to ellipsoidal and typically pear-shaped, a feature which is shared with the two associated holococcolithophores. The coccospheres are normally dithecate (i.e. show inner and outer layers of coccoliths with different morphologies) with dimorphic endothecal coccoliths (Figs. 1, 3). The cell bears two flagellae and a haptonema and the flagellar opening is surrounded by modified coccoliths possessing a spine which is forked at the end (Figs. 1, 2, 4). The elliptical body coccoliths have a corrugated wall with three external flanges (Fig. 3). The central area is formed of numerous radial laths that extend towards the center of the coccolith and are partly joining (Figs. 3, 4). The monomorphic dome-shaped exothecal coccoliths are elliptical with a narrow central depression and slitted walls (Fig. 5). The heterococcolithophore species *Syracosphaera pulchra* contains two chloroplasts and exhibits two flagella.

For quantitative morphological analysis of the heterococcolith stage simple size measurements on body coccoliths have been performed. Data show a strong bi- to multimodality in samples from the fossil record and sediment trap material (Geisen et al. 2002). It was initially unclear whether this variation was due to genotypic or ecophenotypic variability. As cultures became available, these alternative hypotheses were tested by growing cultures under a range of environmental conditions. Morphometrical analyses performed on clonal cultures, revealed a strong unimodality, and no temperature related size variation was observed, indicating that the morphology is under strong genotypic control.

Life-cycles and holococcolith phase differentiation

It has recently been demonstrated that the heterococcolithophore *S. pulchra* independently forms life-cycle associations with two holococcolithophores previously assigned to different genera, *Calyptrosphaera oblonga* and *Daktylethra pirus* (for a review refer to Cros et al. 2000; Geisen et al. 2002). Data here stem from observations both from a phase change observed in culture (*S. pulchra* heterococcolith stage to “*D. pirus*” holococcolith stage) and from combination coccospheres in

Plate 6 *Syracosphaera* spp.

Fig. 1: SEM of a *S. pulchra* coccosphere. This typical specimen displays endothecal and exothecal coccoliths. Coccoliths surrounding the flagellar pole are spine bearing. Water sample, N. Atlantic, off the Canary Islands, R/V Poseidon cruise P233B, station 3.

Fig. 2: SEM of a *S. pulchra* coccosphere without exothecal coccoliths. Water sample, N. Atlantic, R/V *Meteor* cruise 42-4B, station US 1B.

Fig. 3: SEM of *S. pulchra* endothecal coccoliths. The specimen in lateral view shows the typical wall structure with three flanges. Note the lack of exothecal coccoliths. Water sample, N. Atlantic, off Canary Islands, R/V *Meteor* cruise 42-4B, station US 1B.

Fig. 4: SEM of a *S. pulchra* circumflagellar endothecal coccolith. Note the typical central spine with forked end. Water sample, N. Atlantic, off Canary Islands, R/V *Meteor* cruise 42-4B, station US 1B.

Fig. 5: SEM of a *S. pulchra* exothecal coccolith. Water sample, N. Atlantic, off Canary Islands, R/V *Meteor* cruise 42-4B, station US 1B.

Fig. 6: SEM of the holococcolithophore stage of *S. pulchra*. This stage was previously described as “*Daktylethra pirus*” and is referred to as *S. pulchra* HO *pirus*-type. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 69.

Fig. 7: SEM of a *S. pulchra* HO *pirus*-type circumflagellar holococcolith in lateral view. The circumflagellar coccoliths typically have a pointed hood. Note the clear offset between the hood and the base and the presence of perforations in the hood. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 69.

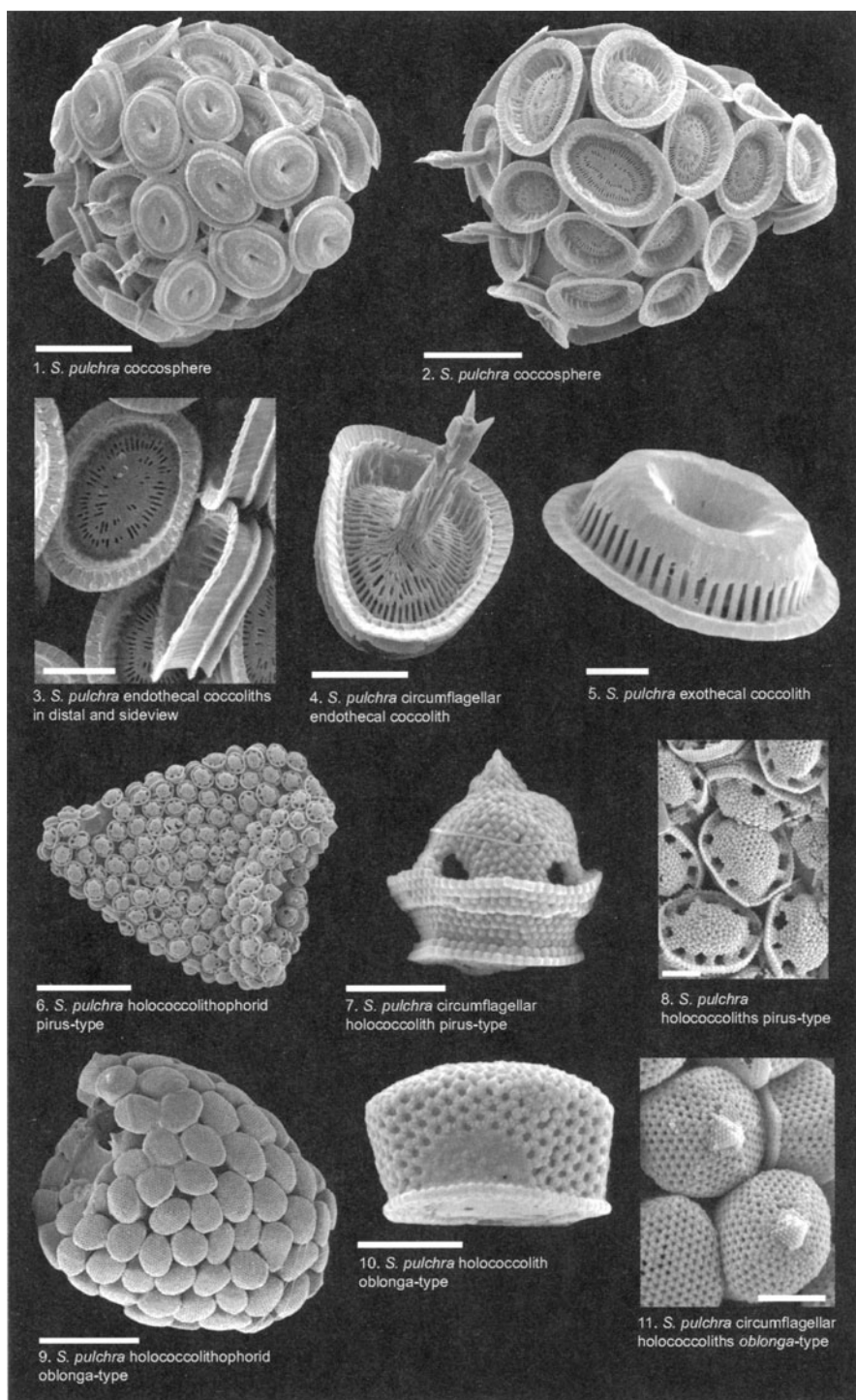
Fig. 8: SEM of *S. pulchra* HO *pirus*-type holococcoliths. The circumflagellar coccoliths typically have a pointed hood. Water sample, N. Atlantic, off Canary Islands, R/V *Meteor* cruise 42-4B, station US 1B.

Fig. 9: SEM of the holococcolithophore stage of *S. pulchra*. This stage was previously described as “*Calyptrosphaera oblonga*” and is referred to as *S. pulchra* HO *oblonga*-type. Water sample, N. Atlantic, off the Canary Islands, R/V *Poseidon* cruise P233B, station 3.

Fig. 10: SEM of a *S. pulchra* HO *oblonga*-type holococcolith. Note the hexagonal arrangement of the calcite rhombohedra and the absence of an offset between the hood and the base. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 69.

Fig. 11: SEM of *S. pulchra* HO *oblonga*-type circumflagellar holococcoliths. The circumflagellar coccoliths typically have a pointed hood. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 69.

Scale bars: Figs. 1, 2, 9: 5 μ m, Fig. 6: 10 μ m, Figs. 3, 4: 2 μ m, Figs. 5, 7, 8, 10, 11: 1 μ m.



water samples. Geisen et al. (2002) have concluded that this is an example of pseudo-cryptic speciation where morphological separation between the species is only visible in the haploid, holococcolith bearing stage. Although they infer that *S. pulchra* probably comprises two biological species it is currently impossible to separate them in the heterococcolith phase and so they introduced the informal terms *S. pulchra* HO pirus-type (Figs. 6–8) and *S. pulchra* HO oblonga-type (Figs. 9–11) until the heterococcolith phase can be discriminated. Re-examination of the morphometrical data from water samples and culture samples suggests that there may be a slight differentiation in the mean size of the endothecal heterococcoliths.

Ecological, biogeographical and molecular separation

The co-occurrence of the two holococcolithophores associated with *S. pulchra* in the same plankton samples has led to speculation about the nature of speciation and – comparable with the case of *Emiliana* – an ecological speciation can be hypothesized. Until now, however, the two *Syracosphaera* species can only be discriminated in their holococcolithophore stage and there is little information on their biogeographical and ecological ranges.

Thirteen clonal strains of *S. pulchra* are currently under molecular investigation to test the hypothesis of a slight differentiation in the mean size of the heterococcoliths between the two potential species. The 18S rDNA genes are strictly identical between the strains, but the first *tufA* DNA sequences show the presence of at least two different types. As for the other species described in this chapter, a comparison between genetic and morphological data will provide a powerful tool to discriminate which subtle morphological character(s) may allow distinction at the species level.

Status of taxon

In this case, heterococcolith morphology is remarkably complex and stable and *S. pulchra* had been regarded as a particularly well-defined species. Detailed study of geological and oceanographic samples however yielded more complex and variable morphological patterns than expected, but these were initially interpreted as essentially noise, i.e. random population-level variation. However, our observations of holococcolith-heterococcolith combination coccospheres and phase changes in cultured strains have indicated strong morphological differentiation in the haploid phase holococcoliths (Geisen et al. 2002). This discovery of cryptic species in *S. pulchra* has now severely challenged the interpretation of *S. pulchra* as a single species with a global occurrence. Until now any testing of the predictions arising from this has been critically dependent on the presence of the relatively rare holococcolithophores and hence biogeographical mapping of the two holococcolithophores involved remains an important target for future research. A further opportunity to solve this challenging problem will be the use of genetic markers to discriminate the two species.

Synthesis

Speciation and divergences (Plate 7)

The set of morphotypes observed in each of the original species and the types of evidence which have lead us to infer that these are genotypically discrete are summarized in Plate 7. Each case is different but the obvious common feature is that taxa which have, with varying degrees of confidence, been regarded as single species prove to consist of a small set of separate species, even including our control species *S. pulchra* which was supposed to be unambiguously well-defined. Clearly this provides strong support for taxonomic splitting and suggests that current estimates of species level diversity in the coccolithophores are likely to be much too low. For biostratigraphy this is very positive since it indicates that fine scale morphological differences can legitimately be used. For Quaternary paleo-ecological work there is strong potential for using the modern morphotypes to refine data retrieval (e.g. Bollmann et al. 2002). Implications for evolutionary understanding rather depend on divergence times and degree of differentiation of the taxa.

In a few cases our research has shown that fine-scale morphovariants of classic species in fact represent fully isolated species, which have diverged in the Pliocene (2–5 Ma) or earlier. For instance *Umbilicosphaera sibogae*, which by some authors has been subdivided into two varieties with alternative hypotheses that they represented ecotypes, life-cycle stages or closely related genotypes (Inouye and Pienaar 1984). Molecular evidence suggests that they have been genotypically independent for more than 5 Ma according to the molecular clock of Sáez et al. (2003). Similarly, in modern *Calcidiscus* three morphotypes have been tentatively distinguished based on size variation and subtle morphological differences (Kleijne 1991, 1993; Knappertsbusch et al. 1997), but there has been uncertainty as to whether they intergrade or are discrete and hence whether they are ecophenotypes (Knappertsbusch 2000; Renaud and Klaas 2001). New data from plankton samples, life-cycle stages, culture studies and molecular genetic work all indicate that they in fact represent discrete species that probably diverged in the Middle Miocene (Geisen et al. 2002; Renaud et al. 2002; Sáez et al. 2003; Quinn et al. 2003). Similarly the *Helicosphaera* species, previously regarded as varieties of *H. carteri*, although only separated by slight morphological variation seem likely to have diverged in the Miocene, based on both geological record and molecular clock data. These results suggest that morphological differentiation can be strongly uncoupled from genetic divergences (see de Vargas et al. this volume, for more discussion on this aspect).

In contrast to these species which diverged long ago a second set of studied cases reveals another level of genotypical variability equally coupled with slight morphological variation. Among them are *Emiliana huxleyi*, *Gephyrocapsa* spp., *Coccolithus* spp., *Calcidiscus quadriperforatus* variants (see Quinn et al. this volume for a discussion on cryptic speciation in *Calcidiscus quadriperforatus*) and *Syracosphaera pulchra*, which reveal a similar pattern of variation, but there is

evidence for more recent divergences. In each case divergence within the last 2 Ma and possibly much more recently seems likely. In these cases it is possible that we are dealing with ecologically separated sub-species rather than completely separate species.

These results indicate that, unless coccolithophores are currently undergoing an exceptional phase of radiative speciation, evolution is a dynamic process continuously producing and eliminating species. This process can be studied by a combination of morphological and molecular genetic methods (see Fig. 5 of de Vargas et al. this volume).

Local adaptation a precursor of ecological speciation?

Paasche (2002) synthesized a range of evidence to suggest that globally distributed species, such as *E. huxleyi* should be regarded as mosaics of locally adapted populations. Evidence includes: (a) genetic fingerprinting data for high levels of genetic recombination, within a haplo-diplontic life-cycle; (b) physiological experiments indicating genetic differentiation within populations and significant variation between environments (Brand 1981, 1982; Fisher and Honjo 1989; Young and Westbroek 1991; Paasche et al. 1996; Paasche 2002); (c) the very broad distribution of such species, occupying improbably wide ranges of habitats, contrasting with narrower ecological tolerances for individual culture isolates. To a certain extent our evidence, that the global species are in fact made up of a few separate species, constitutes an alternative explanation for the phenomenon of individual species having improbably wide geographic ranges and broad ecological tolerances. We did not study variation between strains of our redefined species in detail but our life-cycle data reinforce the importance of sexual reproduction in coccolithophores and preliminary AFLP studies of *Calcidiscus* (Saez unpublished data) confirmed high levels of differentiation within the separate species. So Paasche's model of extensive local adaptation is a possible origin of the numerous relatively recent divergences we have recognized. We suggest that this apparent local specialization may be a key factor for speciation in the coccolithophores, and possibly more generally for the evolutionary success of marine planktic organisms.

Outlook

The current evidence of old speciations, recent divergences and local ecological adaptations, arguably intergrade – indeed it is often difficult to determine which pattern applies in a particular case. Hence a possible model is that local ecological

Plate 7. Outline of intra-specific variation in the CODENET taxa. Heterococcolith phases are illustrated above the holococcolith phases. Boxes indicate the type and the quality of available evidence to support the interpretation. Black boxes – strong data, gray boxes – weak data, white boxes – no data.

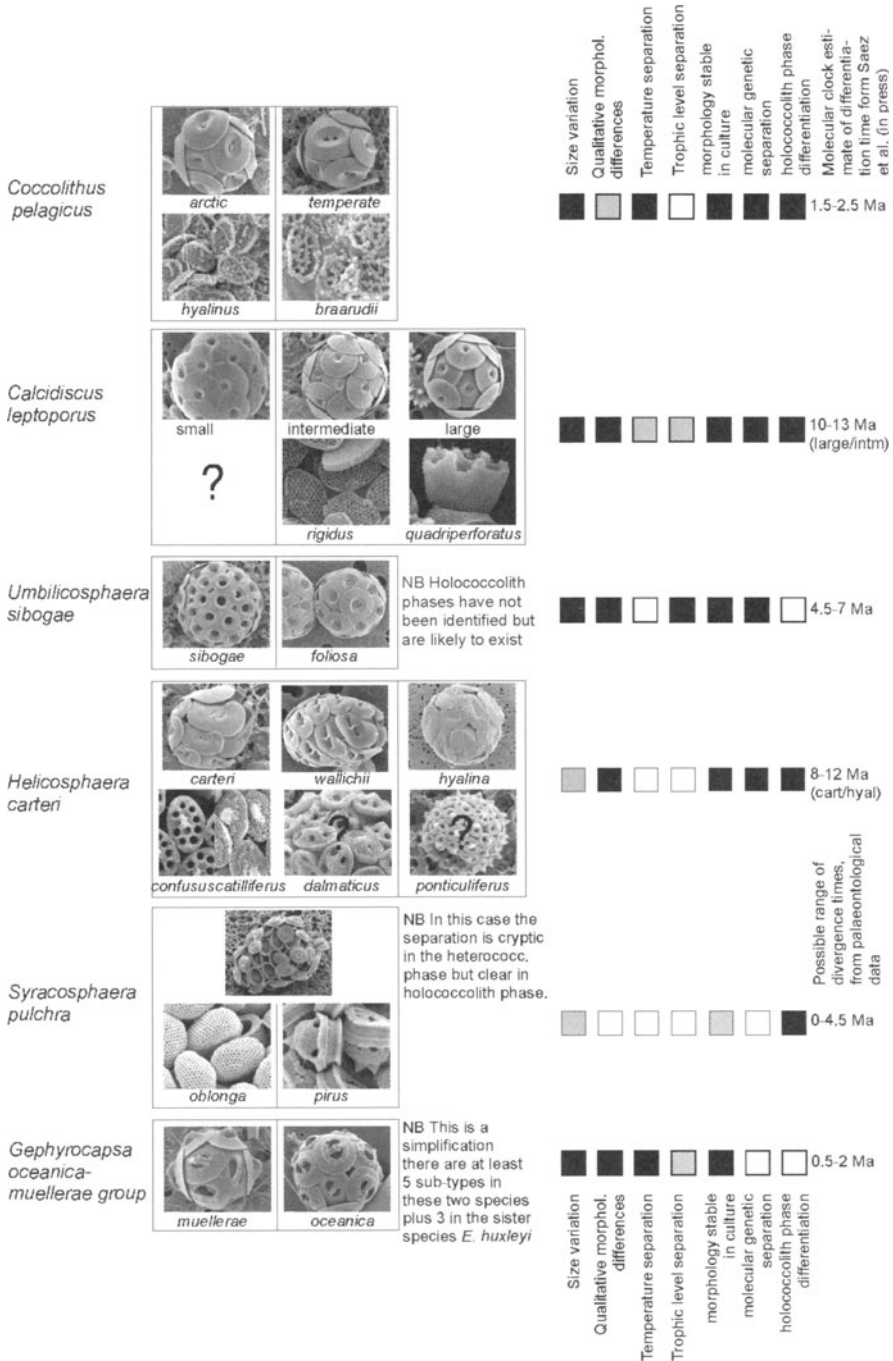


Plate 7.

adaptation leads to continuous evolution of new geographically restricted genetic varieties, which in certain cases differ sufficiently to form discrete sub-species that disperse globally into similar ecological environments. A constant turnover of such sub-species may occur, possibly because of environmental change causing shifts in the extent of the ecological conditions to which they are adapted. If particular sub-species differentiate sufficiently both ecologically and genotypically, then they may diverge into discrete biological species.

A key factor to elucidate the underlying mechanisms of this hypothetical pattern of evolution is the understanding of the coccolithophore life-cycle and reproductive strategy. The presence of chloroplasts provides the algae with a seemingly unlimited source of energy. Unlike in other species however the energy produced is not stored in carbohydrates and fatty acids, but seems to maintain – in the presence of sufficient nutrients – a high biomass level by asexual reproduction (Smetacek 2001). This strategy might well be described as protection by outnumbering of possible predators, an extreme example being a phytoplankton bloom. The apparent decoupling of sexual recombination of genes and reproduction by mitotic fission allows for both rapid (local) differentiation and maintenance of high cell densities. Hence the ability to exchange genes within a population is an important – and in the case of the coccolithophores a long overlooked – tool for adaptation and speciation especially for planktic organisms competing in a rapidly changing environment. As a logical consequence of this strategy it can be hypothesized that coccolithophores, which have lost or not evolved calcification in the haploid stage, might have an evolutionary advantage as this part of the life-cycle only occurs occasionally. Unlike in land plants, where the skeleton serves as a supporting structure in the competition of the chloroplasts for light (Smetacek 2001), this function seems unlikely for these minute marine planktic algae living in near defiance of gravity. Hence mechanical protection against viruses and bacteria trying to enter the cell and against grazers with their mainly acidic stomachs (e.g. copepods) can be hypothesized as a function of coccoliths. However, protection for the relatively short time spent in the haploid stage is not needed and more energy is thus available for reproduction via mitosis. Important future research tasks will therefore focus on life-cycles and reproductive strategies of coccolithophores and will include the identification of possibly ecological triggers to induce phase changes.

Coccolithophores appear to show evidence for both classic models of evolution – phyletic gradualism and punctuated equilibria (allopatric speciation) (Eldredge 1971; Eldredge and Gould 1972; Gould and Eldredge 1977; Pearson 1993; Young and Bown 1994; Benton and Pearson 2001). We have demonstrated here that combining different strands of research can enable us to acquire detailed information on coccolithophore diversity and evolution and to gain further understanding of the underlying processes.

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Coccolith contribution to South Atlantic carbonate sedimentation

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Summary

In order to assess the significance of calcifying organisms for the carbonate budget it is necessary to have reliable estimates of the relative proportion of carbonate production of the different organism groups. In this chapter we firstly review the carbonate distribution patterns of both bulk coccoliths and planktic foraminifera, estimated by means of different carbonate calculation techniques. These studies clearly reveal a variable pattern of carbonate sedimentation and accumulation for planktic foraminifera and coccolithophores, respectively. Whilst coccolith carbonate dominates the oligotrophic gyres of the South Atlantic, carbonate produced by planktic foraminifera is more important in more fertile, mesotrophic to eutrophic areas, such as the equatorial divergence zone. Mass estimates of coccolith carbonate burial in surface sediments of the South Atlantic document coccoliths to be major carbonate contributors in most mid-Atlantic Ridge sediments, exceeding up to 70 wt.-%. In contrast to these oligotrophic areas, they are of lesser importance in sediments accumulating on the continental margins, where they account for only a fifth of the carbonate fraction.

Based on estimates of mean coccolith masses, species-specific coccolith carbonate contributions were calculated for the South Atlantic Ocean. Even though absolute numbers of most species, particularly of *Oolithotus fragilis*, *Rhabdosphaera clavigera*, *Coccolithus pelagicus*, and *Helicosphaera carteri* are many times lower than total numbers of *Emiliana huxleyi*, these subordinate massively calcifying species contribute most to the coccolith carbonate. Despite the large cell numbers generated by *E. huxleyi* and *F. profunda* in the photic zone, as single species they are of minor importance to coccolithophore carbonate production. While absolute accumulation of coccolith carbonate in upwelling regions is estimated to be about three times higher than in the oligotrophic gyres, the latter areas are about 5–10 times larger globally. This means that total accumulation of coccolith-carbonate in oligotrophic areas exceeds by far that of the

upwelling regimes and is therefore of prime importance in global carbonate burial budgets.

Introduction

The marine carbonate system is an important part of the global carbon cycle that controls the atmospheric carbon dioxide (CO_2) contents. Due to the capacity of the oceans to store large amounts of heat and CO_2 – the ocean contains some 60 times the CO_2 of the atmosphere (Berger 1985; Broecker and Peng 1989) – oceans have played a crucial role in atmospheric changes on geological time-scales. Late Quaternary CO_2 records, as revealed by measurements of gas bubbles trapped in ice cores, exhibit pronounced glacial to interglacial variations (Barnola et al. 1987; Petit et al. 1999, see Fig. 1) that seem to be closely coupled to changes in carbon exchange rates within the ocean (Broecker and Peng 1986). Lower glacial CO_2 values appear to be tied to an enhanced transfer of carbon from the surface waters to the deep ocean by higher rates of photosynthesis and biologic productivity (biologic pumping; Berger et al. 1987).

Carbonate production on continental slopes and especially in the pelagic open ocean is almost exclusively planktic. The production of calcium carbonate by planktic organisms in surface waters is difficult to estimate (Balch et al. 2000). Due mainly to the different time-scales involved (days vs. several hundreds of years), only a limited number of studies are available, which compare plankton data directly with spatial patterns of assemblages found in surface sediments (McIntyre and Bé 1967; Samtleben and Schröder 1992; Baumann et al. 2000; Haidar et al. 2000). Calculation of the global ocean carbonate budget includes carbonate production, accumulation, and dissolution, but published estimations of the carbonate budget vary widely. The quantity of biogenic carbonate produced annually in the surface ocean broadly is in the order of 1–1.3 gigatons (Westbroek et al. 1993), from which about 85% dissolves in the water column or at the sediment-water interface. Other authors assume that about 60% of the carbonate accumulates in sediments, whereas the remaining 40% are dissolved (Milliman 1993). Reliable estimates as to the relative proportions of the various pelagic carbonate producers, however, are still sparse (e.g. Giraudeau et al. 2000; Schiebel 2002).

From a quantitative point of view among the most important pelagic calcifying organisms in the present ocean are the coccolithophores, haptophyte algae, which produce minute calcite platelets, the coccoliths (Westbroek et al. 1993). They are significant components of the earth's biogeochemical cycles, owing to their great abundance, fast turnover rates, and their capability to carry out photosynthesis and calcification (Winter and Siesser 1994; Bown 1998). The evolution of pelagic calcareous nannoplankton and planktic foraminifera in the Mesozoic led to the shift in global calcification from the continental shelves towards

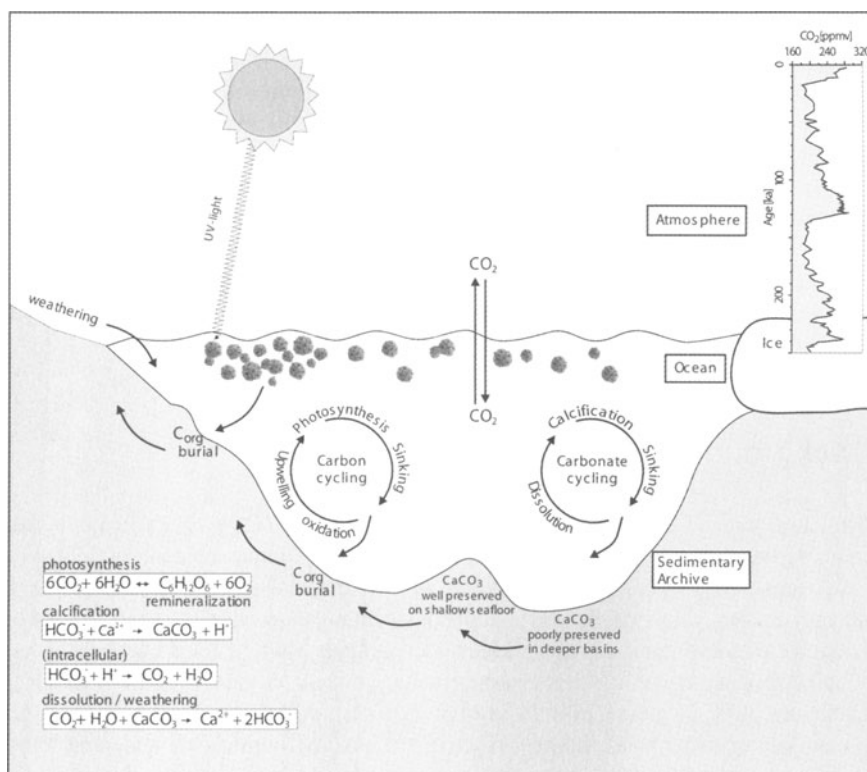


Fig. 1. Schematic representation of the complex role of coccolithophores within the carbon cycle; CO₂ record from the Vostok ice-core by Petit et al. (1999).

important elements in the carbon cycle, shifts in pelagic carbonate sources, production and accumulation may influence atmospheric CO₂ level (Fig. 1). The role of calcite as ballast to sinking particles and thus the efficiency of export productivity has recently been addressed by Buitenhuis et al. (2001).

Consequently, research on coccolithophores may add significantly to a better understanding of the carbon cycle. This paper reviews in some detail the importance of coccolithophores in the carbonate cycle. One has to note that the carbonate budgets in this paper reflect a "geological view", i.e. an integration of several hundreds to thousands of years, as commonly found in the mixed surface sediments of the open ocean, and should not be confused with short-term observations in the water column. Therefore, this paper summarizes and compares estimates of bulk coccolith carbonate contribution and their carbonate proportions with those of planktic foraminifera as recently published for the South Atlantic (Baumann et al. in press; Frenz et al. *subm.*). Another purpose of this paper is to provide carbonate distribution maps of single coccolith species. The study does not solely focus on the CODENET keystone species but includes the most im-

portant taxa that dominate carbonate fluxes in modern marine environments. In particular, numerically important coccolith taxa such as *Calcidiscus leptoporus*, *Emiliania huxleyi*, and *Florisphaera profunda*, are included as well as the carbonate pattern of the less frequent species *Oolithotus fragilis*, *Helicosphaera carteri*, *Umbilicosphaera sibogae*, *Rhabdosphaera clavigera*, *Coccolithus pelagicus*, *Syracosphaera pulchra*, and *Gephyrocapsa oceanica* in the South Atlantic Ocean. All of the carbonate data shown here are based on coccolith counts that were previously published (Baumann et al. 1999; Kinkel et al. 2000; Böckel and Baumann in press; Böckel et al. subm.). Implications of these results for understanding the significance of coccolithophores in the carbonate budget are discussed.

Background information

The study area stretches from about 20°N to 60°S and 60°W to 15°E, including most of the equatorial, central and subpolar South Atlantic Ocean (Fig. 2). We investigated the uppermost centimeter of surface sediment samples collected during various ship expeditions (Appendix 1; deposited at <http://www.coccoco.ethz.ch>). In total, the data set includes 212 samples studied for weight-balanced species-specific coccolith carbonate contents as well as their total sum (data in Baumann et al. in press), and 98 samples for their carbonate grain-size distributions (data in Frenz et al. subm.). Ages of the surface samples are assumed to be Holocene, although ages may vary from decades to several thousands of years depending on the local sedimentation rate. Nonetheless, the surface sediment samples are assumed to provide a reasonable present-day data set as groundwork for extrapolation in the geological past.

Hydrography and production characteristics of the study area

The South Atlantic occupies a key position in the global thermohaline circulation. It is especially important in terms of cross-equatorial transport of warm (> 24°C) surface water to the North Atlantic (Fig. 2), which is balanced by the southward flow of more saline and cooler North Atlantic Deep Water (NADW) to the southern hemisphere (Berger and Wefer 1996).

Trade winds and the westerlies drive the major surface currents in an anticyclonic motion around the oligotrophic Subtropical Central Gyre. Surface water of the South Equatorial Current (SEC) is forming the northern limb of the subtropical gyre (Fig. 2). At about 10°S off Brazil, the SEC splits into two branches, building the southward flowing Brazil Current and the northward flowing North Brazil Current (Peterson and Stramma 1991). The latter contributes to the North Equatorial Counter Current. Its interaction with a northern branch of the SEC

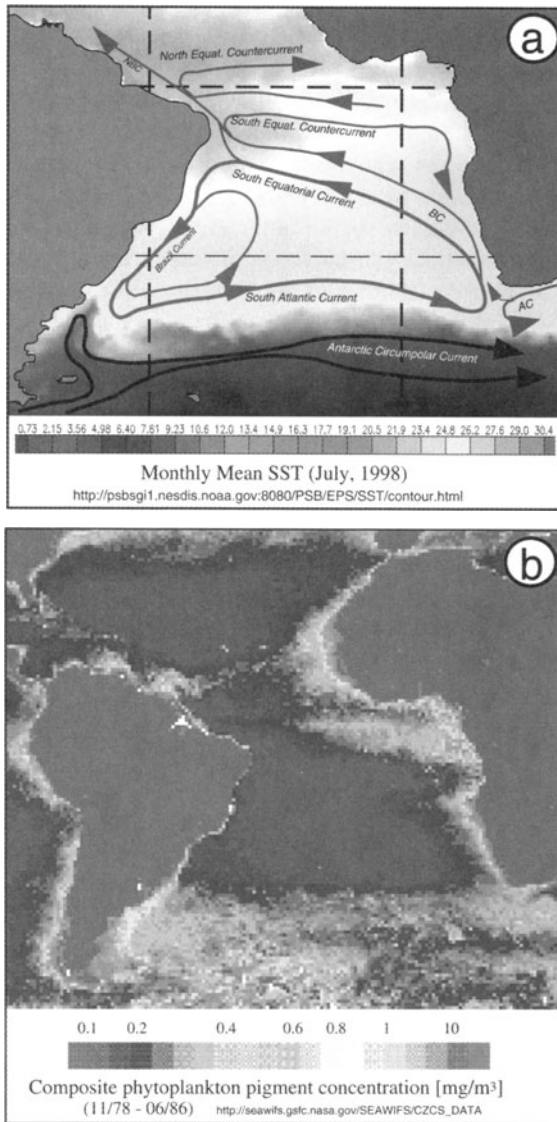


Fig. 2. Map showing (a) a schematic representation of the large-scale upper-level circulation pattern in the South Atlantic (from various sources, see text) plotted on a map of annual mean sea-surface temperatures. (AC – Agulhas Current, BC – Benguela Current, NBC – Northern Brazil Current), and (b) the pigment distribution in surface waters inferred from color scanning data aboard CZCS satellite (a colored version of this figure is deposited at <http://www.coccoco.ethz.ch>).

leads to a strong convergence of water masses in a mixing area at about 3° to 5°N. This results in down-welling of surface waters, which supports the eastward flowing Equatorial Undercurrent (EUC). The contact zone of EUC and SEC forms the equatorial divergence, where upwelling of colder water from around the thermo-cline depth occurs. The source of the upwelling water is essential as it determines the nutrient concentrations in the upper photic zone, where most of the phytoplankton productivity takes place (Monger et al. 1997).

The southern South Atlantic and the adjoining Atlantic sector of the Southern Ocean are both characterized by the eastward-directed Antarctic Circumpolar Current (ACC). At the southern border of the Subtropical Front located around 40°S, the warmer South Atlantic Current waters parallel the cold and nutrient rich ACC in the south (Fig. 2). The boundary between subtropical and subantarctic water might extend regionally over a large area between 30° and 45°S (Smythe-Wright et al. 1998). Another important feature along the warm-water path is the Benguela upwelling region off southwestern Africa, where the Benguela Current transports warm water quite vigorously equatorwards (Berger and Wefer 1996). This region of strong coastal upwelling is one of the main centers of highest ocean productivity (Berger 1989). Despite the relatively small area involved, eastern boundary upwelling plays an important role in global biogeochemical cycles and processes affecting the interior of the ocean (e.g. Reimers et al. 1992). According to Walsh (1991), continental margins may be responsible for cycling as much carbon as the open ocean. Here, the primary production rate can reach $> 100 \text{ g C m}^{-2} \text{ a}^{-1}$ and is between 50 and $100 \text{ g C m}^{-2} \text{ a}^{-1}$ in the equatorial divergence zone. In contrast, primary production is generally $< 50 \text{ g C m}^{-2} \text{ a}^{-1}$ in the oligotrophic gyres (Berger 1989). Therefore, variations in upwelling strength and nutrient content of upwelled waters driving the productivity in the surface layer might lead to large-scale changes in biologic pumping to the deep-sea with possible impacts on CO_2 levels.

Methods

Grain-size analyses

The quantification of different carbonate portions based on grain-size investigations applied here was already introduced by Robinson and McCave (1994) and McCave et al. (1995) and is described in detail by Frenz et al. (subm).

Upon a separation into sand, silt and clay sub-fractions a representative split of the bulk silt (BS) of 2–3 g was briefly dispersed in 0.05 % calgon solution and the grain-size distribution was measured using a Micromeritics SediGraph 5100. Afterwards, the remaining BS was rejoined with the measured portion, dried, weighed, and then the carbonate content was removed by stepwise addition of hydrochloric acid. Subsequently, this carbonate-free silt (S_{cf}) fraction was washed to neutral pH, dried, weighed and then measured with the SediGraph.

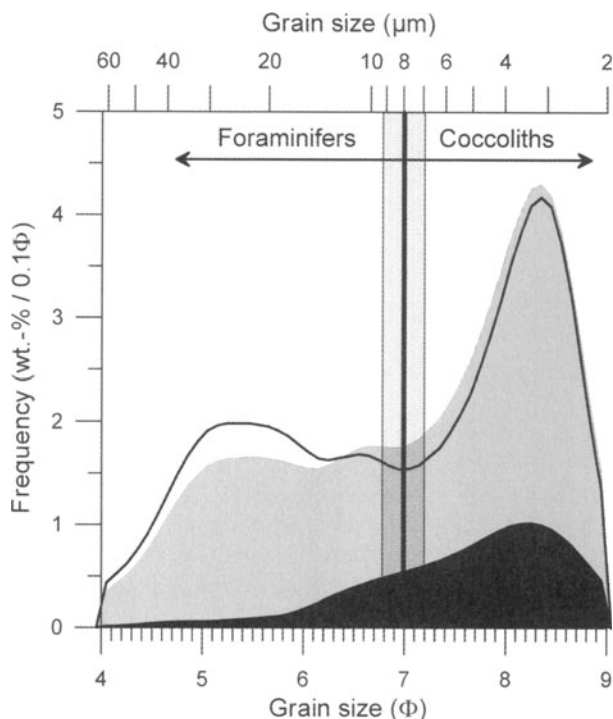


Fig. 3. Distribution of the silt fraction in surface sample GeoB 6410-1 to illustrate the calculation of the carbonate silt grain-size distribution from both the given BS (bulk silt) and S_{cf} (carbonate-free silt) distribution as well as known carbonate content of the silt fraction (here 79 %). The black curved line is the total BS distribution (sum of all classes 100 %). The black shaded area is the relative S_{cf} distribution sum of all classes = (100 % – 79 %). The gray shaded area is the relative CS (carbonate silt) distribution that is given by the difference of both lines in each size class.

(about 62.5 to 2 μm). The carbonate portions of sand, silt, and clay were re-scaled to 100% of the sub-fraction weights.

From the two grain-size distributions analyzed (BS and S_{cf}) and the known bulk silt carbonate content the grain-size distribution of the carbonate silt (CS) was calculated. The unreduced data were converted into 50 equal size classes from 4–9 Φ calculated (Fig. 3). The relative CS size distribution is the difference between the absolute BS and the relative S_{cf} size distribution (rel. CS = %tot. BS – %rel. S_{cf} in each size class). A general minimum observed in the CS grain-size distributions at about 8 μm led to the distinction that carbonate particles with equivalent spherical diameters > 8 μm mainly consist of planktic foraminifera and their fragments. Calcareous particles < 8 μm are coccoliths and occasionally dinoflagellate cysts.

Table 1. Mean sizes (in μm) of 15 to 230 measurements per species and sample used for estimates of species-specific carbonate masses.

Site	Sample type	Latitude	Longitude	Depth (m)	<i>C. leptoporus</i> small	<i>C. leptoporus</i> large	<i>C. leptoporus</i> interm.	<i>C. pelagicus</i>	<i>E. huxleyi</i>	<i>F. profunda</i>	<i>G. oceanica</i>	<i>H. carteri</i>	<i>O. fragilis</i>	<i>R. clavigera</i>	<i>S. pulchra</i>	<i>U. sibogae</i>
<i>Equatorial Divergence</i>																
WA 8	Sed. trap	0.14	-23.45	3744	4.01	8.26	6.71		3.27	3.06	4.45	9.23			5.63	5.27
Geob 1901	Surf. Sediment	-0.82	-16.24	2879	3.70				2.50	4.00		13.00			4.60	5.70
Geob 4412	Surf. Sediment	5.72	-44.36	3763			6.50		2.50	2.30		5.70			5.30	4.30
<i>Central South Atlantic</i>																
Geob 1415	Surf. Sediment	-15.53	-11.58	3116		8.31	5.78		3.21	2.86		10.00		9.49	6.47	5.28
Geob 1903	Surf. Sediment	-8.68	-11.84	3161	4.18	7.69	5.89		3.03	3.04		8.86		8.05	6.10	3.99
Geob 3807	Surf. Sediment	-30.75	-13.20	2534			6.40		3.40	2.90		9.70			5.70	5.70
Geob 5136	Surf. Sediment	-19.37	-12.67	3227			5.90		2.40	4.90		7.30			5.50	4.60
<i>Benguela Upwelling</i>																
NU 2	Sed. trap	-29.2	13.11	2516		9.36	6.59	12.70	3.28	2.68	5.11	9.22	7.12	8.83	5.08	4.92
WR 2	Sed. trap	-20.05	9.15	599	3.81		5.98		3.16	3.44	4.77	9.62			6.46	5.40
Geob 1710	Surf. Sediment	-23.43	11.70	2983			6.51	12.91			5.01	10.13			6.22	5.15
Geob 3603	Surf. Sediment	-35.13	17.54	2851			5.56	10.61	3.37	2.87	5.50	9.69			4.77	5.56
Geob 3725	Surf. Sediment	-23.32	12.37	1980			5.30		3.60	3.40		10.30			4.60	6.00
<i>Southern Ocean</i>																
Geob 2714	Surf. Sediment	-43.87	-57.99	2361			6.70		3.60	4.50		8.20			6.30	7.30
Geob 6418	Surf. Sediment	-38.43	-21.54	4126		9.17	6.50					10.00		9.73		5.81

Coccolith data and analyses

Total coccolith numbers and size measurements provide the essential variables for the estimation of coccolith carbonate. Preparation of all sediment samples followed a dilution/filtering technique described by Andruseit (1996). Generally, a small amount of sediment was weighed and suspended in tap water. After ultrasonic treatment the suspension was split in the rotary splitter and then filtered onto polycarbonate filters (0.4 μm pore size). The filters were then dried and small filter cuts were examined under the SEM allowing the quantification of coccoliths per gram dry sediment. Qualitative and quantitative examination of the coccoliths was carried out on a Scanning Electron Microscope (Zeiss DMS 940A) at magnifications of 3000 or 5000x.

Coccolith numbers were then converted into coccolith-carbonate contents. A calculation of species-specific coccolith carbonate contribution to the bulk sediment was done following the method given in Young and Ziveri (2000), which is based on estimates of the mean carbonate mass of the various species. This approach has already been applied in sediment trap samples (e.g. Broerse et al. 2000; Giraudeau et al. 2000; Sprengel et al. 2000) and includes the shape of a coccolith type, the density of calcite, and – most important – the average length of coccoliths. To optimize carbonate calculations size ranges of the most common species were measured in a number of surface sediments and sediment traps (Table 1).

Coccolith distribution and bulk coccolith carbonate – previous work

Information on the global distribution of deep-sea carbonate, which is the main component of deep-sea sediments above the lysocline, is already available (Archer 1996). Estimates of the relative proportions of the various pelagic carbonate producers are, so far, only available for the South Atlantic (Baumann et al. in press; Frenz et al. subm.). These results are summarized and compared in the following chapter. In addition, coccolith counts, as the basis for species-specific carbonate calculations, are provided by various investigations (Baumann et al. 1999; Kinkel et al. 2000; Böckel and Baumann in press; Böckel et al. subm.) and are briefly presented first.

Distribution patterns of coccoliths in South Atlantic and Southern Ocean surface sediments

Bulk coccolith contents range from 0–83 $\times 10^9 \text{ g}^{-1}$ sediment in the South Atlantic and Southern Oceans (Fig. 4a). Highest coccolith numbers were detected in the central South Atlantic, whereas lowest coccolith contents were found in

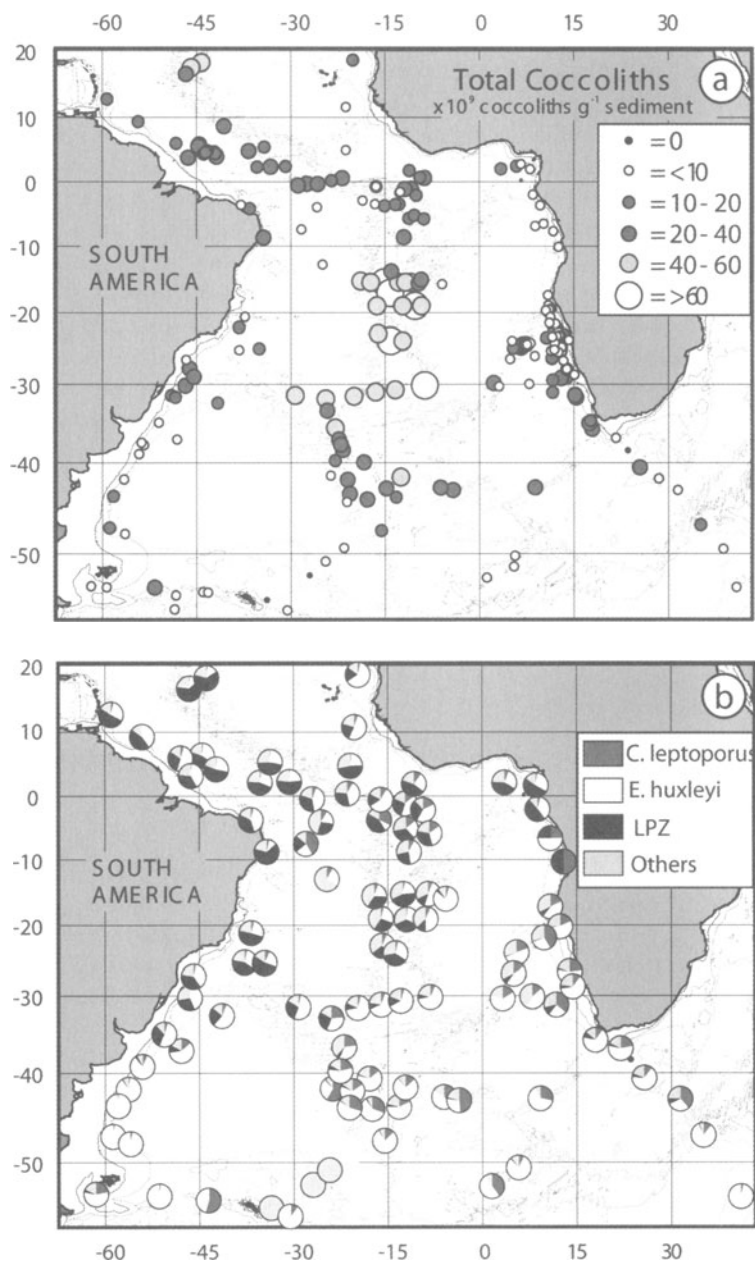


Fig. 4. Maps of the South Atlantic surface sediments showing **a)** absolute numbers of total coccoliths, and **b)** pie charts of the relative abundances of *C. leptoporus*, *E. huxleyi*, the lower photic zone species (*F. profunda* and *G. flabellatus*) as well as other species (modified from Böckel et al. subm.).

the Southern Ocean, in the deep-sea basins, on the continental margins of southern South America, and off the western coast of central Africa. Highest coccolith numbers in the central South Atlantic have been interpreted as a result of minimum dilution. Additionally, the majority of the coccolithophore species seems to be well adapted to low nutrient conditions, so that the highest diversities are found in the subtropical central gyres (Böckel et al. *subm.*). On average the numerically dominant species encountered in surface sediments from the South Atlantic and Southern Ocean is *E. huxleyi* (Fig. 4b). This ubiquitous species exhibits highest abundances in sediments deposited underneath the Benguela upwelling domain (> 50%) and south of 32°S, especially on the continental margin off South America (> 80%). Accordingly, minimum values are encountered in sediments north of 32°S latitude especially in the central South Atlantic (mostly < 30%). The distribution of the lower photic zone (LPZ) taxa, comprising *F. profunda* and *G. flabellatus*, basically delineates the opposite trend. In sediments between 20°N and 30°S LPZ taxa are mostly more abundant than *E. huxleyi*. In the western equatorial Atlantic between 20° and 2°N, the easternmost equatorial Atlantic and along the South American continental margin as far south as 30°S they can even dominate the coccolith assemblages (Fig. 4b). Highest percentages (> 40%) of the most abundant LPZ species, *F. profunda*, are found in equatorial sediments between 10°N and 5°S and along the southern South American continental margin. Analogously, nannoliths of this species appeared only in low numbers (< 15%) south of 40°S.

Carbonate contributions of coccoliths and planktic foraminifera in the South Atlantic

Two previous studies on surface sediments independently reveal a variable pattern of carbonate contributions mainly derived from planktic foraminifera and coccolithophores (Baumann et al. *in press*; Frenz et al. *subm.*).

Calculation of coccolith carbonate based on estimates of mean species carbonate mass indicate that coccoliths are by far the main contributors to the carbonate in the oligotrophic gyres of the South Atlantic, often exceeding 70 wt.-% (Baumann et al. *in press*). In contrast to the mid-Atlantic Ridge, where coccoliths dominate the carbonate fraction (60–70%), they are of minor importance in sediments accumulating on the continental margins, only accounting for about a fifth of the carbonate fraction. In contrast, carbonate derived from planktic foraminifera increased considerably in more fertile, mesotrophic areas, such as the equatorial divergence zone (Fig. 5). However, in terms of weight-balanced carbonate input foraminifera rarely exceed 50 wt.-%. Foraminiferal carbonate is much more affected by dissolution than coccolith carbonate and the dissolution begins already far above the calcite lysocline. This was suggested to be due to the relatively long-term exposure at the sediment/water interface leading to increased carbonate dissolution of the surface sediments (Baumann et al. *in press*).

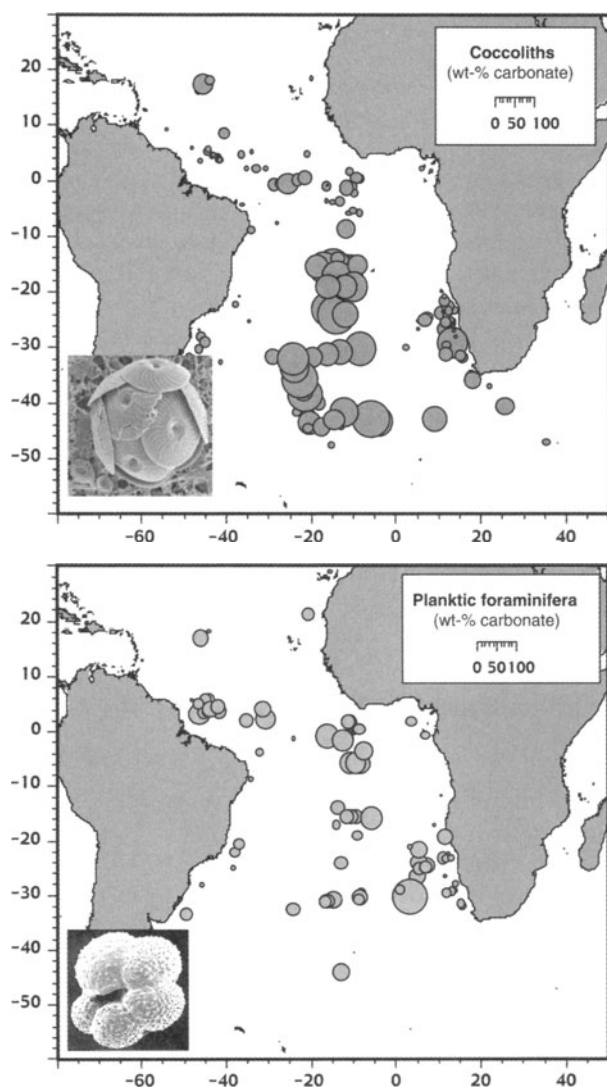


Fig. 5. Maps of weight-balanced carbonate contents of coccoliths (upper figure) and planktic foraminifera (lower figure) in surface sediments of the South Atlantic (from Baumann et al. in press). For foraminifera carbonate, the carbonate of the $> 63 \mu\text{m}$ fraction was geochemically measured. In fact, carbonate input of the different organism groups is generally dominated by coccoliths in the oligotrophic gyres of the South Atlantic, whereas carbonate derived from planktic foraminifera increased in more fertile, mesotrophic areas, such as the equatorial divergence zone.

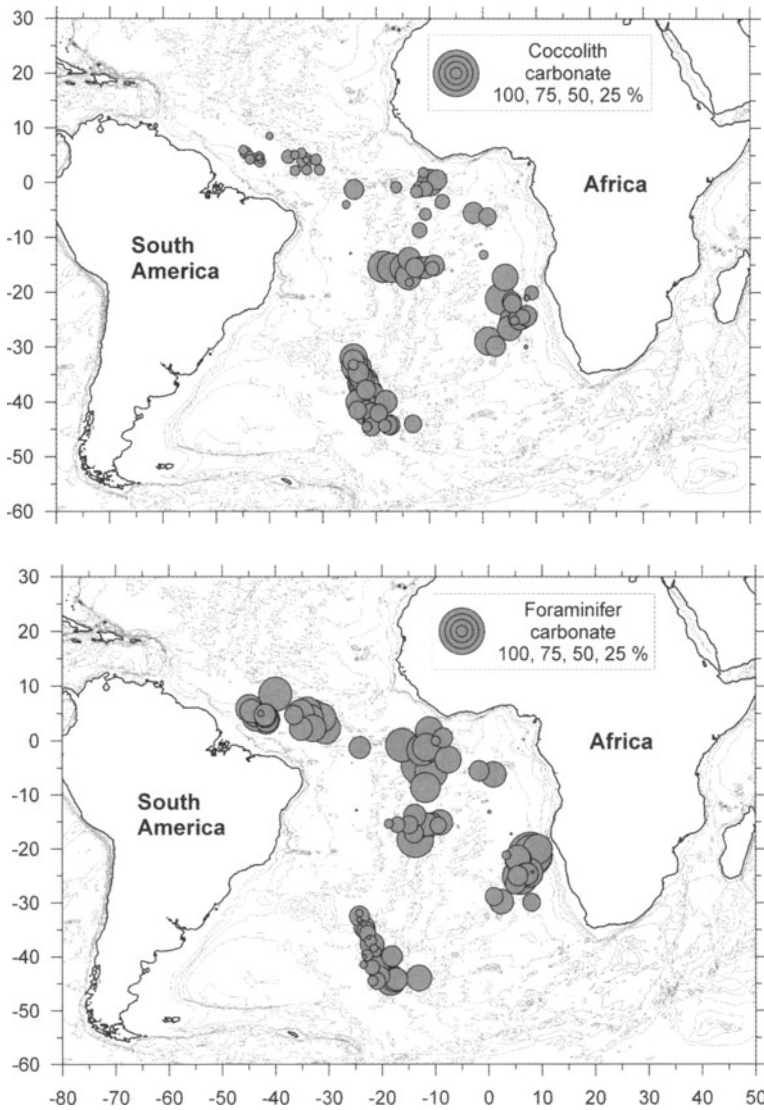


Fig. 6. Regional distribution of weight-balanced (upper figure) ESD $< 8 \mu\text{m}$ carbonate mainly produced by coccoliths, and (lower figure) equivalent spherical diameters (ESD) $> 8 \mu\text{m}$ carbonate, basically derived from planktic foraminifera, (modified from Frenz et al. *subm.*). These data indicate that coccoliths are the major carbonate contributors in most mid-Atlantic Ridge sediments, whereas highest foraminiferal carbonate contributions are found below the relatively fertile, mesotrophic waters of the equatorial divergence.

In addition, aragonitic pteropods are of regional importance at the continental margin of the western South Atlantic, whereas other calcareous organisms only supply a minor proportion of calcium carbonate to the sediments.

Grain-size investigations indicate a striking bimodal distribution of the calcareous silt fraction (CS) in surface sediments of the South Atlantic. These populations are clearly separated by a low frequency size interval in the silt fraction (see Fig. 3) ranging from 10–7 μm (average 8 μm = 7 Φ). A similar low has previously been found in a grain-size analysis of fine fractions (Paull et al. 1988). This allowed quantification both of a coarse mode and a fine peak and, thus, a quantification of different carbonate portions (Frenz et al. *subm.*). SEM observations of the sub-fractions indicate that carbonate particles with equivalent spherical diameters larger than 8 μm mainly consist of planktic foraminifers and their fragments. Calcareous particles smaller than this are coccoliths and occasionally dinoflagellate cysts (Frenz et al. *subm.*). On the basis of this division the regional variation of the contribution of foraminifers and coccoliths to the carbonate budget of the sediments were calculated (Fig. 6).

Planktic foraminifera carbonates display the highest carbonate contribution to the sediments in the equatorial upwelling area and off SW-Africa. Coccoliths dominate in the central and southern part of the South Atlantic. Thus, the regional distribution of foraminifer and coccolith carbonate (Fig. 6a, b) confirms the findings based on mass estimates of bulk coccolith carbonate and shows that mesotropical regions (equatorial province) are the favored habitats of foraminifers, whereas coccoliths production dominates in oligotrophic regions.

Comparison of the methods

A comparison between the two methods used here and previously by Baumann et al. (*in press*) indicate generally correlated results (Fig. 7) and, therefore, these data are thought to be a good approximation both of the planktic foraminifera and of the coccolith carbonate contributions.

Differences in the estimates, however, are revealed by deviations of the correlation lines from a slope of one (Fig. 7a). This was attributed to the presence of relatively small foraminifer fragments that are not recognized in the sand fraction, but are detected by means of silt grain-size analyses. Taking these silt-sized fragments into account, the sand-fraction analyses (y axis) underestimates the foraminiferal carbonate content by about 10–20 % (Frenz et al. *subm.*). However, the total coarse carbonate could also have included pteropods and minor amounts of various other calcareous particles.

The coccolith carbonate calculated on the basis of mass estimates of individual species compared to the carbonate content of the size fraction < 8 μm is in rather good agreement (Fig. 4b). It has to be noted that the grain size fraction < 8 μm refers to spherical particles and not coccolith length, i.e. the general coccolith size in general is much larger, since they are disk shaped. However, deviations of ± 20 % are common. Besides general analytical errors of both

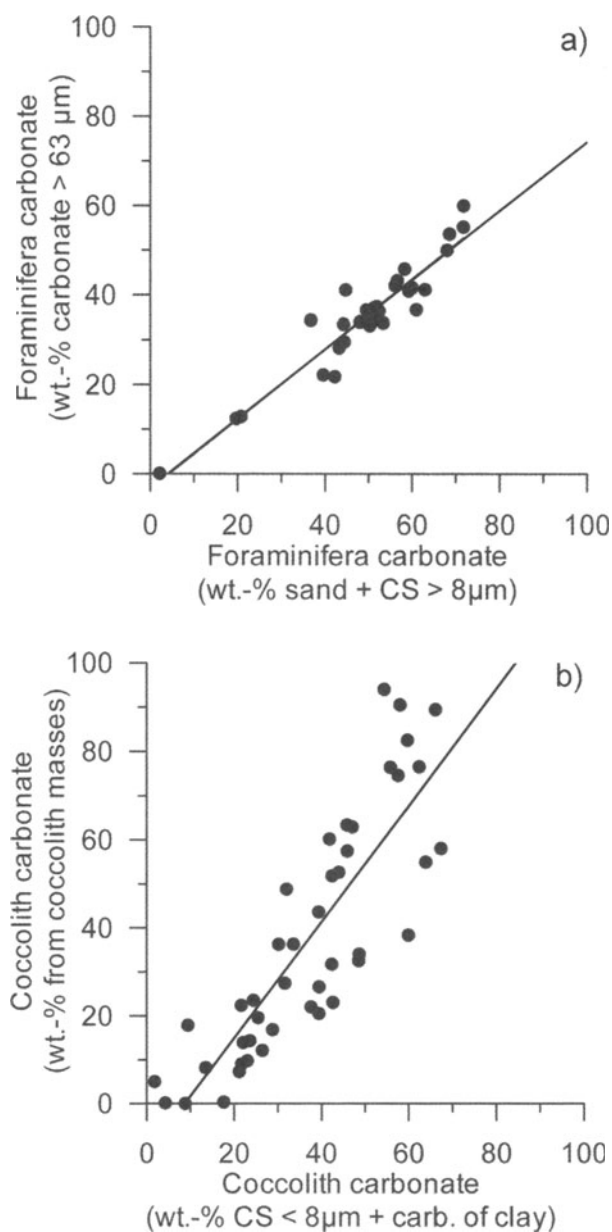


Fig. 7. Comparison of the measurements derived by the two different carbonate estimations used in this study indicating a fairly good correspondence of both **a.** the foraminifera carbonate calculations ($r^2=0.9$) and **b.** the coccolith carbonate calculations ($r^2=0.74$).

methods, mainly the extrapolation of individual coccolith masses to the huge number of individuals is held responsible to cause the observed deviations. Even if considerable care is taken, the calculations may result in relatively large errors of up to 50 % (Young and Ziveri 2000). Nevertheless, the data given here seem very reasonable in comparison to the total carbonate data, and, therefore, are expected to be a good approximation of the coccolith-carbonate content.

Carbonate distribution patterns of the most common coccolith species – New results

In the following sections, we present biogeographical coccolith carbonate distributions of the numerically abundant taxa, such as *Calcidiscus leptoporus*, *Emiliania huxleyi*, and *Florisphaera profunda*, and also show the carbonate patterns of subordinate, but strongly calcified species. The latter include *Oolithotus fragilis*, *Helicosphaera* spp., *Umbilicosphaera sibogae*, *Rhabdosphaera clavigera*, and *Coccolithus pelagicus*. All together these species mostly contribute > 90% of the coccolith carbonate in all of the investigated surface sediments (data in Appendix 2; deposited at <http://www.coccoco.ethz.ch>). To get a full range of the CODENET keystone species we have also included maps of *Syracosphaera pulchra* and *Gephyrocapsa oceanica*.

Species-specific coccolith carbonate distribution

Calcidiscus leptoporus

Calcidiscus leptoporus is present in surface sediments underlying various oceanographic regimes (Böckel et al. subm.; Ziveri et al. this volume). It is especially abundant south of the Subtropical Convergence (STC) and in the Benguela upwelling region, whereas it is rarest in the western equatorial Atlantic and in sediments underlying open-oceanic subtropical waters.

The mapped *C. leptoporus* carbonate distribution (Fig. 8) shows a rather marked maximum at mid latitudes (30° to 45° S). The species' carbonate contribution rise up to > 50 wt.-% in the mid-Atlantic Ridge sediments of the central South Atlantic. Obviously, *C. leptoporus* dominates by far the carbonate fraction in this area (> 50 % of the total carbonate). In addition, as a carbonate producer this species is moderately important in the other areas of the South Atlantic (central South Atlantic, off SW Africa), but never exceeds 30 wt.-%, whereas it only plays a minor role at the continental margin off South America and in the equatorial divergence zone.

Comprising a mean of 35% of the total coccolith carbonate which is about 14% of the total carbonate content, *C. leptoporus* is the most important coccolith carbonate producer to South Atlantic surface sediments. Its conspicuously high

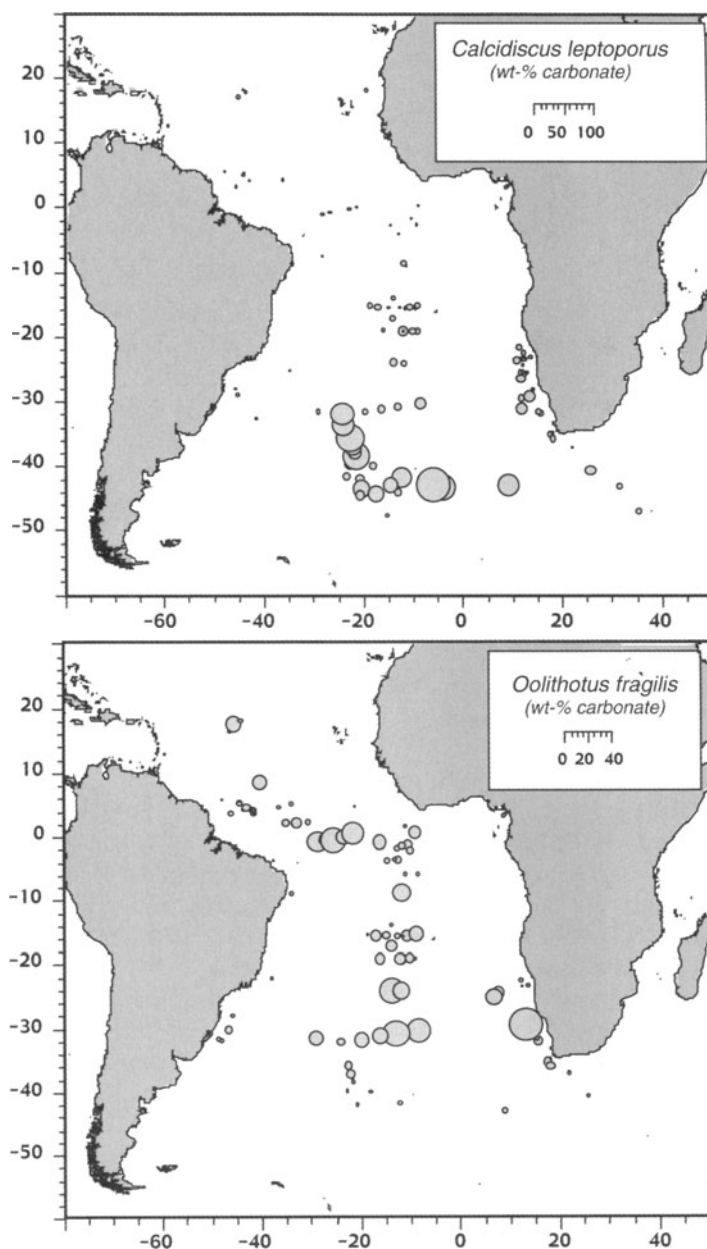


Fig. 8. Carbonate distribution patterns of major carbonate contributors *Calcidiscus leptoporus* and *Oolithotus fragilis*. Even though *C. leptoporus*, and particularly of *O. fragilis* coccoliths are only common, these relatively massive species are the most important contributors to the total coccolith carbonate.

abundance in more fertile areas, may also be the result of selective dissolution leading to a relative increase in its robust coccoliths. *Calcidiscus leptoporus* is known to be one of the most dissolution resistant species (Schneidermann 1977). In eutrophic regimes, more organic carbon is produced. Its microbial breakdown leads to the acidification of the water-column and upper sediment layer, which can be responsible for increased carbonate dissolution (e.g. Dittert et al. 1999).

Oolithotus fragilis

Surprisingly, *Oolithotus fragilis* also contributes significantly to the carbonate content in surface sediments (Fig. 8). This species shows a rather widespread distribution but only low abundances in the study area (Böckel et al. subm.). Its carbonate input ranges from > 20 wt.-% in sediments of the equatorial Atlantic and central South Atlantic to < 1 wt.-% south of 35°S and off South America. The mid-Atlantic Ridge sediments contain *O. fragilis* derived carbonate, which generally comprise between 4 and 28 wt.-% of the total sediment (mean of 15% of total coccolith carbonate).

These relatively high carbonate contributions of *O. fragilis* are encountered in various oceanographic regimes. This is in good agreement with other studies that did not reveal any distinct ecological affinities of *O. fragilis* (Böckel et al. subm.). It appears that *O. fragilis* is a relatively important contributor of carbonate in sub-tropical meso- to oligotrophic areas.

Emiliania huxleyi

Emiliania huxleyi is the dominant coccolith species in surface sediments of the South Atlantic (Böckel et al. subm.; see Fig. 5) and exhibits highest abundances in sediments deposited underneath the Benguela upwelling and south of 30°S.

Accordingly, carbonate derived from *E. huxleyi* is highest in sediments south of about 30°S latitude but seldom exceeds 10 wt.-% (Fig. 9). In tropical and subtropical areas its carbonate input is even less than 3 wt.-%. Thus, *E. huxleyi* only plays a minor role as a supplier of carbonate to the sediments, at least in the Equatorial and South Atlantic. On average, *E. huxleyi* contributes about 7% to the coccolith carbonate and only about 3% of the total carbonate in the study area. The low contribution to coccolith carbonate results mainly from the small size and delicate structure of its coccoliths. This is rather surprising, since this species was considered to be the world's major producer of calcite and one of the largest single carbonate sinks in oceanic carbonate cycling (Westbroek et al. 1993). Large-scale blooms, that mainly consist of *E. huxleyi*, are regularly observed in the northern North Atlantic (e.g. Holligan et al. 1993; Brown and Yoder 1994) but also in the southwestern South Atlantic (Gayoso 1995). These blooms are thought to influence global climate by affecting the inorganic carbon system of the seawater (e.g. Buitenhuis et al. 1996). The present results, however, indicate that production of CaCO_3 by species other than *E. huxleyi* could have greater effects on marine and atmospheric cycling of CO_2 .

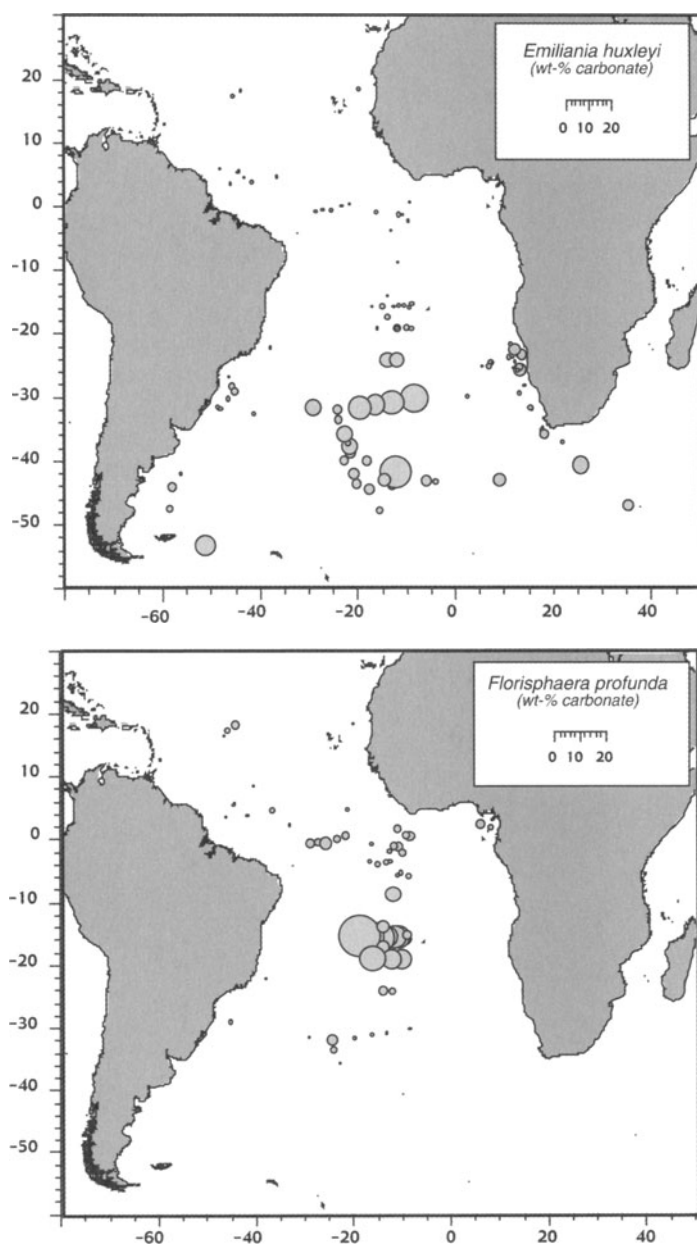


Fig. 9. Carbonate distribution patterns of the numerically dominant species *Emiliana huxleyi* and *Florisphaera profunda*. Because these species produce comparatively small nannoliths, they are minor carbonate contributors.

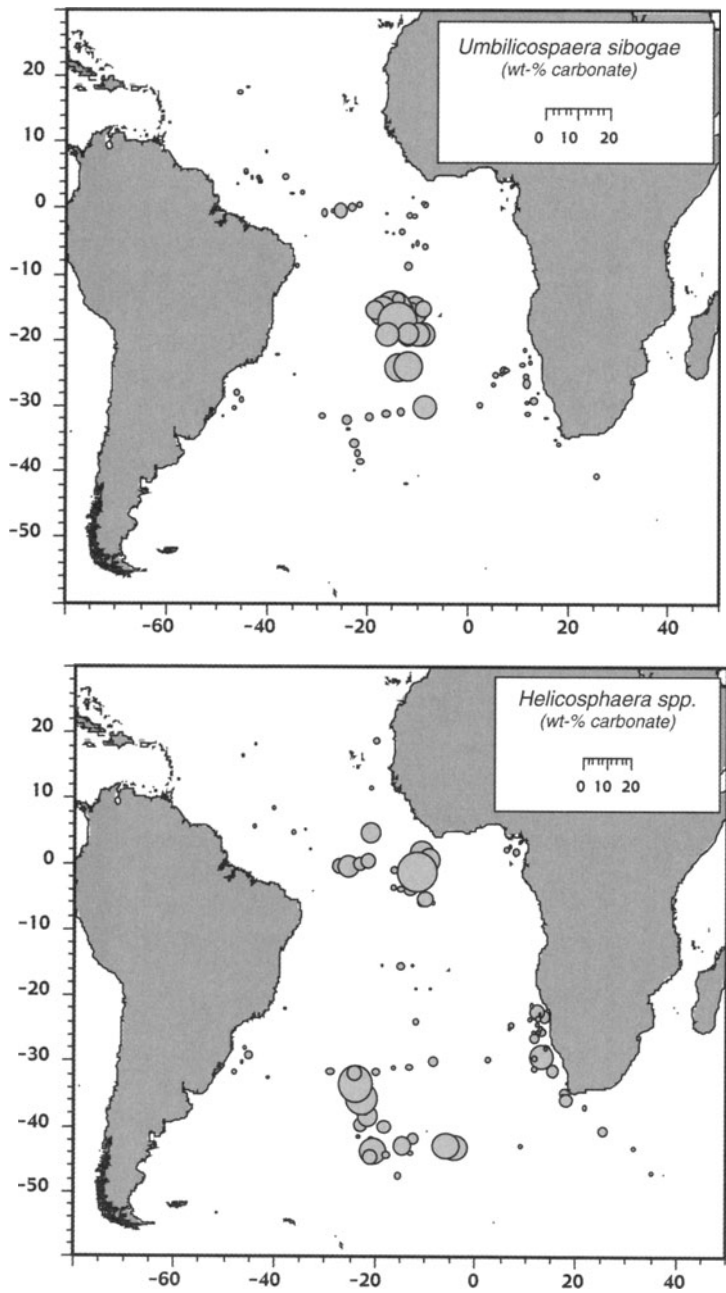


Fig. 10. Map of the weight-balanced carbonate distribution patterns of *Umbilicosphaera sibogae* and *Helicosphaera* spp.

Florisphaera profunda

In tropical to subtropical areas between 20°N and 30°S the deep-dwelling *Florisphaera profunda* are often more abundant in surface sediments than *E. huxleyi* and sometimes even dominate the coccolith assemblages (Böckel et al. subm.).

However, highest carbonate contents of *F. profunda* of up to > 10 wt.-% are only found in mid-Atlantic Ridge sediments between 10° and 20°S (Fig. 9). This species contributes moderately (up to 5 wt.-%) to the bulk carbonate in the equatorial divergence zone. Thus, as for *E. huxleyi*, carbonate derived from this numerically important species only plays a subordinate role in the carbonate budget of the sediments in the Equatorial and South Atlantic. On average, *F. profunda* contributes about 6% to the coccolith carbonate and only about 2.5% of the total carbonate in the study area.

Umbilicosphaera sibogae

Coccoliths of *Umbilicosphaera sibogae* are widely distributed. Highest numbers are encountered mainly in tropical and sub-tropical latitudes from 8°S to 32°S (Böckel et al. subm.). Moreover, a well-defined abundance maximum in the South Atlantic corresponds rather closely to the oligotrophic gyre (Ziveri et al. this volume).

Maximum carbonate contributions derived from *U. sibogae* of up to > 10 wt.-% are found in sediments of the mid-Atlantic Ridge in the central South Atlantic Ocean (Fig. 10). Outside this area, its carbonate contribution to the sediment always was less than 2 wt.-%. On average, *U. sibogae* makes up 7.5% of the coccolith carbonate and contributes about 3.2% to the total carbonate of the studied samples. A differentiation into the two varieties/species, *U. sibogae sibogae* and *U. sibogae foliosa* (see Geisen et al. this volume), has not been made in all of our analyses and thus their carbonate contents were calculated and mapped together.

***Helicosphaera* spp.**

Helicosphaera species exhibit a widespread distribution, although mostly low abundances were recorded (Böckel et al. subm.). However, these strongly calcifying species are quite important with respect to coccolith carbonate production (Fig. 10). Their carbonate content is of regional importance in the equatorial and central South Atlantic. In these areas, the calculated carbonate contribution amounted to up to 10 wt.-% of the total sediments. Some of the samples from the Benguela Upwelling region were characterized by medium-high carbonate contributions of up to > 5 wt.-% (Fig. 10). In contrast, *Helicosphaera* spp. only scarcely contribute to the carbonate in the sub-tropical gyres.

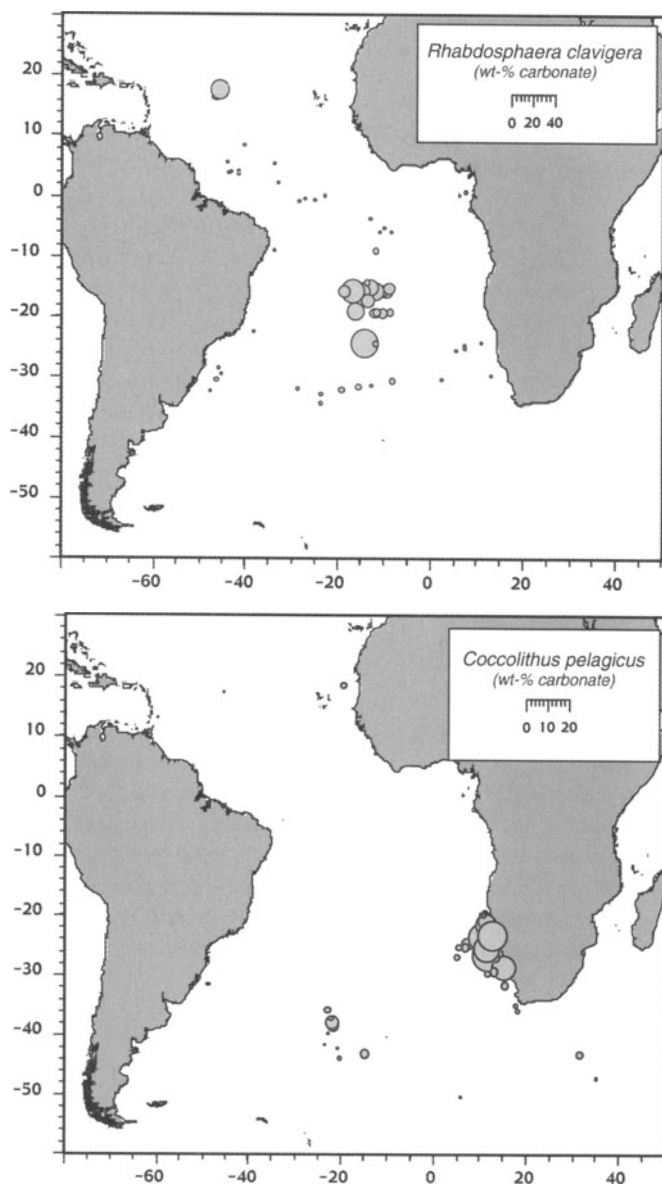


Fig. 11. Map of the weight-balanced carbonate distribution patterns of *Rhabdosphaera clavigera* and *Coccolithus pelagicus*. Even though their absolute numbers are low, these massive species are locally important coccolith carbonate producers.

Our data includes three subunits of this genus (*H. carteri*, *H. wallichii*, and *H. hyalina*, most likely species (see also Geisen et al. this volume). These most likely have discrete ecologies and therefore different abundance patterns with respect to their carbonate input. They have, however, not been distinguished in most of our analyses.

Rhabdosphaera clavigera

Rhabdosphaera clavigera coccoliths only occur in low abundances in surface sediments of the South Atlantic (Böckel et al. subm.). Therefore, its relatively high carbonate input in sediments of the central South Atlantic (10° to 25°S) is rather surprising. Their weight-balanced carbonate contents rise up to > 20 wt.-% in the mid-Atlantic Ridge sediments of the central South Atlantic (Fig. 11). These dissolution resistant coccoliths are a significant component of the carbonate fraction in this area, whereas they only play a minor role off eastern Africa and in the equatorial divergence zone. Nevertheless, *R. clavigera* carbonate comprises more than 9% of the total coccolith carbonate and about 4% of the total carbonate content in samples of the studied area.

Coccolithus pelagicus

In general, coccoliths of *Coccolithus pelagicus* are only sporadically encountered in surface sediments of the South Atlantic. Biogeographical data primarily reflects the clear abundance maxima in the northern North Atlantic (Ziveri et al. this volume). Despite low absolute numbers, this strongly calcifying and dissolution resistant species is a locally important coccolith carbonate producer. A single large *C. pelagicus* coccolith contains about 100 times the carbonate mass of one *E. huxleyi* placolith (see Young and Ziveri 2000). Thus, in sediments of the Benguela Upwelling it accounts for up to > 10 wt.-% of carbonate (Fig. 11). *C. pelagicus* also contributes moderately to the surface sediment carbonate contents south of 35°S, where up to 5 wt.-% were calculated.

Syracosphaera pulchra

Although coccoliths of *Syracosphaera pulchra* occur only scarcely in surface sediments, they exhibit a widespread distribution. They play only a minor role as a supplier of calcium carbonate to the sediments, but with a relatively distinct abundance pattern (Fig. 12). Carbonate contents of up to a maximum of 4 wt.-% were calculated in the mid-Atlantic Ridge sediments, whereas otherwise less than 1 wt.-% of carbonate were recorded.

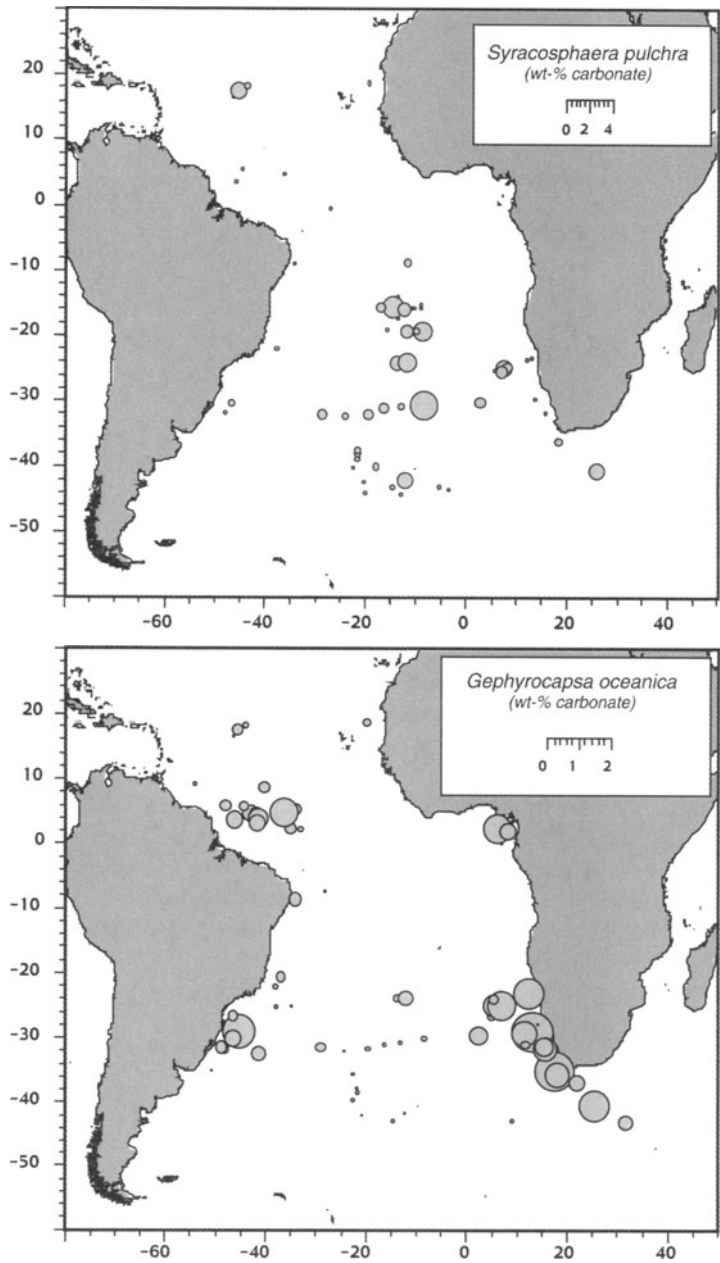


Fig. 12. Map of the weight-balanced carbonate distribution patterns of *Syracosphaera pulchra* and *Gephyrocapsa oceanica*.

Gephyrocapsa oceanica

Gephyrocapsa oceanica appears in moderate abundances in sediments of the southeastern Atlantic (Böckel et al. subm.). Slightly elevated numbers were also found on the continental slope off South America. However, in all these sediments the calcium carbonate contributions of *G. oceanica* remain quite low, never exceeding 2 wt.-% (Fig. 12). In addition, the species is essentially absent in the central oligotrophic gyre.

Coccolith carbonate in surface sediments of the South Atlantic

The distribution patterns of coccolith carbonate clearly reflects frontal systems of the South Atlantic. The particular oceanic environments are recognized by characteristic coccolith carbonate assemblages (Fig. 13) that can already be distinguished by their coccolith numbers (Böckel et al. subm.; Ziveri et al. this volume). In particular, the carbonate distribution of *C. leptoporus* shows a rather marked dominance in the oligotrophic mid-latitudes. Carbonate distributions of *U. sibogae* and *R. clavigera* correspond rather closely to the oligotrophic gyre of the central South Atlantic. Relatively high carbonate contents of *O. fragilis* are encountered in various oceanographic regimes. The carbonate content derived from other species such as *H. carteri* and *C. pelagicus* are of local importance. Their carbonate distribution patterns indicate that these species have affinities towards nutrient-enriched environments and/or cooler surface waters. Whereas *H. carteri* carbonate is common in the equatorial Atlantic *C. pelagicus* contributes to the surface sediment carbonate contents of the Benguela Upwelling. The carbonate distribution of each of these species is much more distinct than the numerical occurrences of their coccoliths.

Even though absolute numbers of *O. fragilis*, as well as particularly of *C. pelagicus*, *R. clavigera*, and *H. carteri*, are many times lower than total numbers of *E. huxleyi*, these subordinate, strongly calcifying species are at least locally important with respect to coccolith carbonate burial in sediments. In contrast, despite large cell numbers generated by *E. huxleyi*, it is of minor importance to coccolithophore carbonate production (Fig. 13). Carbonate contributions of the numerically important deep dwelling *F. profunda*, as for *E. huxleyi*, only play a relatively minor role and are limited to the sediments in the Equatorial and South Atlantic. These results may, at least partly, derive from selective dissolution of fragile coccoliths and a relative increase of more massive and dissolution-resistant coccoliths. The disappearance of coccoliths from the sediment occurs through a gradual, selective removal of ultrastructural elements, a process that may provided a means of subdividing the carbonate compensation zone (e.g. Schneidermann 1977). Initial dissolution is detectable in sediments deposited as shallow as 3000 m, with only a few species showing obvious signs of corrosion. In contrast, the coccolith assemblage immediately overlying the carbonate compensation depth (CCD) is reduced to comparatively well preserved, resistant coccoliths such as *C. leptoporus*, *Helicosphaera* spp., *U. sibogae*, and *C.*

pelagicus. Thus, selective dissolution may slightly bias the species-specific coccolith carbonate contents but does not massively influence the mapped contributions of the various species. This is so because most of the dissolved coccoliths are from small and delicately structured species with very small carbonate masses. With the exceptions of the numerically important *E. huxleyi* and *F. profunda* they can even be ignored in carbonate estimations of sediment traps where their coccoliths are well preserved (e.g. Sprengel et al. 2000; Giraudeau et al. 2000).

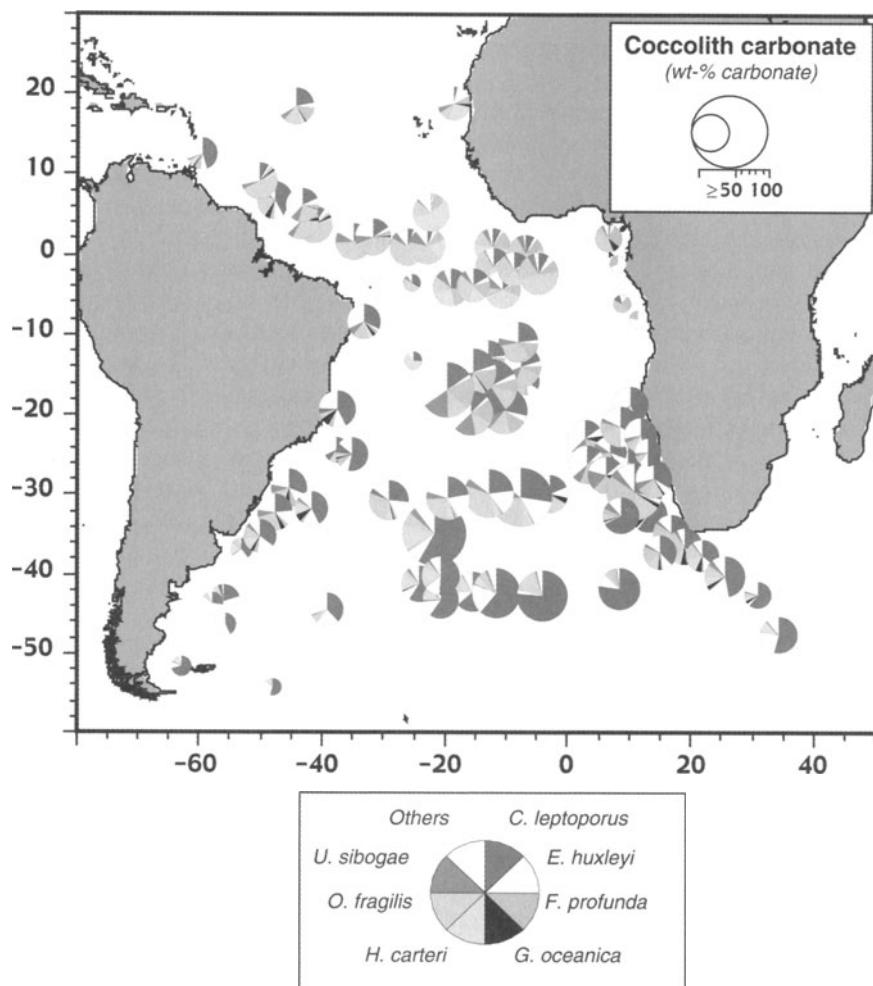


Fig. 13. Summary of the different species-specific coccolith carbonate contributions in surface sediments of the South Atlantic.

Discussion and outlook

Factors influencing coccolith carbonate distribution in surface sediments

The actual carbonate content of deep-sea sediments is controlled by a complex interplay of production in overlying surface waters, dilution by non-carbonate phases and, of course, by dissolution (e.g., Dittert et al. 1999; Henrich et al. in press). The latter is indicated by a relatively high proportion of unidentified carbonate in the sediments that is suggested to most probably come from broken and slightly dissolved specimen (Baumann et al. in press). This finding is linked to the fact that degradation of organic carbon in sediments promotes the dissolution of calcium carbonate. A number of studies have demonstrated that calcite dissolution, driven by metabolic CO_2 produced within the sediments, forms a significant part of the early diagenesis of sedimentary calcite even above the lysocline ("supralysocline dissolution"; e.g., Archer et al. 1989; Hales and Emerson 1997). Recent dissolution studies suggest a strong influence of supralysocline dissolution with respect to aragonite and carbonate in the high productivity areas of the eastern South Atlantic (Gerhardt and Henrich 2001; Volbers and Henrich 2002). Milliman et al. (1999) have suggested that considerable dissolution (perhaps as much as 60%) occurs even in the upper 500–1000 m of the ocean, however, it is not clear if all carbonate producing organisms are affected in a similar manner.

Another factor influencing the carbonate content is dilution by non-carbonate phases, which also contribute to the bulk sedimentation rates. In particular, continental slopes are often dominated by various amounts of terrigenous sediments, discharged by nearby rivers, as well as relatively high contents of both organic carbon and biogenic opal (e.g., Schneider et al. 1997; Arz et al. 1999). In addition, winds are known to carry tremendous amounts of lithogenic dust from dry African source areas to the deep sea (e.g., Tiedemann et al. 1989; Ruddiman 1997; Stuut et al. 2002). Therefore, the proportion of carbonate is highest on the mid-ocean ridge, where dilution as well as dissolution are at their minimum.

A carbonate budget (production vs. loss) can, however, only be estimated on the basis of carbonate accumulation rates as a product of carbonate content, dry bulk density, and sedimentation rate. Fragmentary information on sedimentation rates in some of the areas was obtained from sediment cores nearby indicating a rather high variation of sedimentation rates. They range from less than 1 cm ka^{-1} on the mid-Atlantic Ridge to up to $> 10 \text{ cm ka}^{-1}$ on the Amazon fan and the Benguela Upwelling (see Table 2). In the next section these data are used to obtain a more detailed understanding of sedimentation processes.

Table 2. Location and bulk sedimentation rates available for surface-sediment of the South Atlantic.

Core	Latitude	Longitude	Sedim. rate (cm ka ⁻¹)	Reference
<i>Equatorial Atlantic</i>				
GeoB1041-1	-3.48	-7.59	4.7	Bickert and Wefer 1996
GeoB1101-4	1.67	-10.98	2.7	Bickert 1992
1117-2	-3.81	-14.89	3.8	Bickert and Wefer 1996
GeoB1118	-3.50	-16.43	2.7	Bickert 1992
GeoB1112-3	-5.78	-10.74	2.8	Bickert 1992
A180-073	0.17	-23	1.3	Vogelsang et al. 2001
GeoB1523-1	3.82	-41.62	2.1	Rühlemann et al. 1999
GeoB4412	5.72	-44.36	3.0	Bleil et al. 1997
<i>Central South Atlantic (Subtropical Gyre)</i>				
INMD-115BX	-17.64	-16.21	2.3	Sarnthein et al. 1994
GeoB1034-1	-21.72	5.43	2.0	Bickert and Wefer 1996
<i>Walvis Ridge</i>				
GeoB1211-1	-24.47	7.54	1.2	Bickert and Wefer 1996
GeoB1214-1	-24.69	7.24	1.3	Bickert and Wefer 1996
<i>Benguela Upwelling</i>				
GeoB 1710	-23.26	11.41	5.8	Kirst 1998
PC12	-22.16	12.32	14.2	Summerhayes et al. 1995
GeoB 1023	-17.09	10.6	25.0	Gingele and Dahmke 1994
ODP Site 1079	-11.56	13.24	>20.0	Berger et al. 2002
GeoB 3603	-35.07	17.32	3.1	Esper et al. 2000
<i>Southern Ocean</i>				
PS1754-1	-46.77	7.61	1.7	Frank et al. 1996
PS1756-6	-48.90	6.73	1.0	Frank et al. 2000
PS1768-1	-52.59	4.46	12.2	Frank et al. 2000
PS2082-3	-43.21	11.75	2.5	Frank et al. 1996
PS2498-1	-44.09	-14.14	3.9	Asmus et al. 1999

Coccolith carbonate accumulation

We roughly compare the role of sedimentation/accumulation rates on the burial of coccolith carbonate in the South Atlantic. In view of the coarse spatial resolution (Table 2), it was not feasible to estimate carbonate accumulation for the

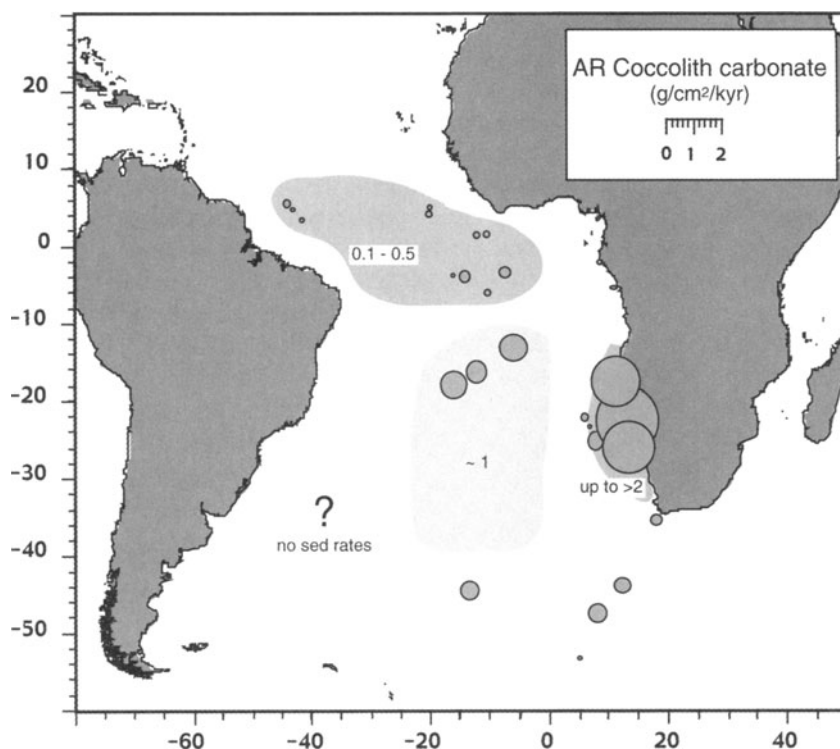


Fig. 14. Coccolith carbonate accumulation rates based on bulk coccolith carbonate contents, available sedimentation rates (see Table 2), and mostly mean dry bulk densities of surface-sediments. High rates are found at the continental margin off Namibia, whereas accumulation rates are still moderate in the oligotrophic pelagic realm. Numbers in the different areas indicate mean accumulation rates of coccolith carbonate.

whole Equatorial and South Atlantic. General trends, however, can be inferred from the available data (Fig. 14). By comparing the end members of sedimentation rates observed, a simple calculation can be made. In the oligotrophic gyres where sedimentation rates are about $1\text{--}2\text{ cm ka}^{-1}$, and coccoliths account for up to 80 % of the total carbonate, about $0.8\text{ g cm}^{-2}\text{ ka}^{-1}$ coccolith carbonate are accumulating. In contrast, high sedimentation rates up to 25 cm ka^{-1} are observed in the upwelling areas off SW-Africa. Here the contribution of coccoliths to the carbonate fraction is much less. Assuming an average carbonate content of 50% in these sediments, to which coccoliths contribute about 20%, an accumulation of $2.5\text{ g cm}^{-2}\text{ ka}^{-1}$ of coccolith carbonate can be estimated. Accordingly, accumulation of coccolith carbonate in upwelling regions is about three times as much as in the oligotrophic gyres (Fig. 14). Although high coccolith carbonate rates are found along the continental margin off Namibia, accumulation rates are still moderate and show little variation in the oligotrophic pelagic realm. Con-

sidering the size of high versus low productivity areas, integrated coccolith burial in the South Atlantic is estimated to be 5–10 times smaller in the upwelling regimes than the oligotrophic gyres.

Moreover, it is obvious that the eutrophic areas are characterized by smaller coccoliths, whereas the oligotrophic areas show a higher abundance of larger forms (Böckel et al. *subm.*). This partly explains the differences observed in the patterns of coccolith carbonate and in the total carbonate budgets, and might be a result of coccolith calcification rate response to nutrient availability. It has been documented by Paasche and Brubak (1994), that calcification increases in *Emiliana huxleyi* under phosphorous limitation. Although, this effect has not been studied in detail for other coccolithophore species, the results presented here seem to indicate that this observation may well be extrapolated to species-specific carbonate production in nutrient rich and nutrient poor areas of the South Atlantic.

Role of coccolithophores within the carbon cycle

Particulate inorganic carbon is produced by pelagic calcifying organisms in the upper water layers of the ocean. All this calcium carbonate produced annually in the surface ocean sinks to the deep-sea and is partly dissolved or partly stored in the geological archive. This phenomenon, known as the carbonate pump, is an essential part of the global carbon cycle and exerts a major influence on climate (Westbroek et al. 1993). Marine pelagic biota also influence the global climate by means of the organic carbon pump. Carbon dioxide is removed from the atmosphere and the upper water layers to deeper waters by photosynthesis and the subsequent sinking of particulate organic carbon. Only 0.1% of the organic matter produced is preserved in the geological archive, the rest being remineralized. In addition, the removal of dissolved inorganic carbon and alkalinity from the upper mixed layer and its partial regeneration in the deep-sea by calcification, sinking, and partial dissolution of particulate inorganic carbon is effectuated via the carbonate pump. It is assumed that about 15–20% of the calcium carbonate production is transferred into the sedimentary archive (Westbroek et al. 1994; Milliman and Droxler 1996).

The flux of coccolith calcite to deep ocean sediments is recognized to be an important factor in determining the exchange of CO₂ between the oceans and the sediments (Sarmiento et al. 1988). In addition, it is suggested that carbonate production by coccolithophores has a large effect on increasing sinking velocities and thus particle export from the photic zone to the deep-sea (Buitenhuis et al. 2001). Since coccolith carbonate production is coupled to photosynthesis and organic carbon production, a substantial amount of organic carbon is associated to the carbonate flux of coccolithophores. This is more efficiently exported to the deep ocean compared to sinking particles that do not contain carbonate. Therefore, Buitenhuis et al. (2001) concluded that coccolithophore carbonate and organic carbon production and sedimentation on geological time-scales act

as a sink for atmospheric CO₂, which is in contrast to the general assumption that calcification and the associated alkalinity changes are a source for this essential greenhouse gas. Mapping of coccolith carbonate accumulation rates is therefore important and might provide valuable insights in areas where efficient export of inorganic and organic carbon is encountered. These findings help to determine key regions that should be studied in order to address changes in these export fluxes and their impact on the global carbon cycle in the past.

Coccolith calcification is thought to be influenced by changes in the global carbon cycle, and it has been proposed, that calcification rates of coccolithophores will decrease under increasing atmospheric CO₂ concentrations as we currently experience due to the burning of fossil fuel and other human activities (see Rost and Riebesell this volume).

Our study demonstrates, that changes in the species composition of coccolithophores might have a much larger influence on the global carbonate production, then the reduction of calcification. For long-term reconstructions of variations of global carbonate budgets, it is of particular interest to compare traps and sediment records from low to high productivity regimes and to determine coccolith carbonate flux and Late Quaternary accumulation rates.

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Biogeography of selected Holocene coccoliths in the Atlantic Ocean

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Summary

In this chapter we present a revision of the biogeographical distribution of five coccolithophorid species (*Coccolithus pelagicus*, *Calcidiscus leptoporus*, *Helicosphaera carteri*, *Syracosphaera pulchra* and *Umbilicosphaera sibogae*) and the genus *Gephyrocapsa* in the Atlantic Ocean. The mapping is based on surface sediment samples. Each of the taxa considered here constitutes an unambiguous morphological group ideal for rapid low taxonomic resolution analysis of assemblages, which is a tempting strategy for ecological and paleoecological analysis of assemblages. However, in each case recent research has indicated that these broad taxa are in fact composed of several discrete species, or sub-species. The clearest example is *C. pelagicus*, with discrete morphotypes in sub-Arctic and temperate upwelling areas. For *Gephyrocapsa* and *Umbilicosphaera* the separation is less obvious but still unambiguous. Species separation is manifestly essential to understanding the biogeography of these taxa. For *H. carteri* and *S. pulchra* the mapped distributions are relatively straightforward and we do not yet know how they relate to the recently proven genotypic variation within the taxa.

At high latitudes temperature and productivity belts parallel each other and the effects are difficult to distinguish. At lower latitudes however, the effects are more clearly separable - it is for instance obvious that *S. pulchra* shows a warm water low productivity preference whilst *H. carteri* shows a warm water higher productivity distribution. In particular there are several cases where distribution patterns in the North and South Atlantic are strikingly different. These include the absence

of *C. pelagicus* in the sub-Antarctic; the much higher abundance of *C. leptoporus* in temperate South Atlantic than North Atlantic; much higher abundance of *U. sibogae* var. *sibogae* in the oligotrophic South Atlantic than the North Atlantic. The *Calcidiscus* and *Umbilicosphaera* patterns are more symmetric, since the North and South Atlantic show broadly similar sets of environments in terms of temperature, salinity, productivity and macronutrients (nitrate, phosphate and silicate). Obvious possible hypotheses are that the populations in the two oceans are sufficiently separated to have evolved slightly different ecological tolerances or that an additional factor, such as a trace element is responsible for the distribution contrasts. More generally we suspect that the comparably broad coccolithophorid biogeographic zones in all oceans and the absence of obvious vicariance in coccolith species distributions may have prevented recognition of significant contrasts between oceans, although such contrasts may provide key clues for interpreting past temporal shifts in assemblages.

Introduction

Meaningful integration of paleobiology into studies, and especially modeling, of global change requires detailed understanding of the ecology of key organisms. As part of the European CODENET (Coccolithophorid evolutionary biodiversity and ecology network) project we investigated the prime constraints on coccolithophorid ecology and the ecological significance of biodiversity within the coccolithophores. This study represents a multidisciplinary analysis of the ecology of coccolithophorid species, including comparison and synthesis of information from physiological studies, biogeography, seasonal succession, and paleontological response to global change (see also Geisen et al. this volume). This is intended both to maximize the paleoecological information retrieval from these species, and to enhance understanding of the ecology of the coccolithophores as a group. This allows critical interpretation of the coccolith record and modeling of the role of coccolithophores within the global carbon cycle.

Coccolithophores are the predominant group of calcifying phytoplankton. As a result they have the best fossil record of all phytoplankton and play a unique role in the global carbon cycle. The outstanding fossil record is provided by open ocean pelagic sedimentation, where coccolith/nannofossil oozes have been the most widely developed facies over the last ca. 200 Ma. Assemblages in surface sediments can be closely related to the living coccolithophorid communities as has been shown for the North Atlantic when extensive datasets are available (Baumann et al. 2000). Nonetheless, comparison of nannofossil compositions between plankton and sediment assemblages is not straightforward, because: (a) a plankton sample represents one instant in time and one water depth whilst a sediment sample typically is an averaged record of several hundred years production from a geographic area of at least a few 100 km² (as a result of variable lateral transport during deposition) and (b) during deposition the smaller and more delicate species are liable to be lost. Hence for interpretations of the fossil record, surface sediment

assemblages may represent a more useful calibration link with oceanographic conditions than do plankton samples. They are ideal for exploring what types of patterns are genuinely preserved in the fossil record. Therefore mapping and understanding surface assemblages is an essential key to interpreting temporal changes of coccoliths in the geological past.

Within the auspices of the CLIMAP project reconstructions (CLIMAP 1976, 1981, 1984), extensive study of the biogeographic distribution of coccoliths in surface sediments was carried out (Geitzenauer et al. 1977 and references therein). However, since CLIMAP, only limited progress has been made in refining global coccolithophorid biogeography. In this chapter we present a revision of the biogeographical distribution of the CODENET keystone taxa (*Coccolithus pelagicus*, *Calcidiscus leptoporus*, *Helicosphaera carteri*, *Syracosphaera pulchra*, *Umbilicosphaera sibogae* and the genus *Gephyrocapsa*) in the Atlantic Ocean. The mapping is based on surface sediment samples. These selected species are solution resistant (Schneidermann 1977) and have a very well known stratigraphic record.

The contour maps presented here are based on syntheses of data from quantitative assemblage analyses of about 500 surface sediment samples, some new and some previously published (Appendix 1, see <http://www.coccoco.ethz.ch/ziveri>).

Species concepts

A problem of using data from diverse sources is that species concepts are liable to vary dependent on the authors, the date of the study, and the methodology used. Hence consistent data can only be obtained for well-defined broad morphotypes, i.e. at a low taxonomic resolution. Each of the six taxa used here reflects this, they have well-defined morphologies and are clearly separated from any other taxon. We can for example be confident that all the authors will have recorded all *C. pelagicus* specimens as *C. pelagicus* and will not have included any other taxa. However, recent studies (e.g. Bollmann 1997; Sáez et al. 2003; Quinn et al. this volume) have shown that many apparently globally distributed coccolithophorid species may be composed of two or more genotypically discrete species (or sub-species) with often subtle morphological differences but rather strong ecological differences. This applies to all the species studied here, as summarized by Geisen et al. (this volume). Because of the heterogeneity of the datasets used here we are unable to differentiate these morphotypes. Hence our analysis is perhaps better seen as a first stage study identifying the large scale patterns and allowing, for each species-group, the identification of key areas for more detailed future studies. Literature data on morphotype distributions has been added to selected maps and detailed results from a set of South Atlantic samples analyzed by one of us (BB) are shown in additional maps.

Nomenclatural revisions proposed by Geisen et al. (2002) and Sáez et al. (2003) add extra complications. Names such as *H. carteri* and *U. sibogae* are now ambiguous. In the traditional meaning *U. sibogae* included two varieties *sibogae* and *foliosa*. *U. foliosa* is now regarded as a separate species, so the modern meaning of *U. sibogae* is more restricted. Where necessary we use “s.l.” (sensu

lato) or s.s. (*sensu stricto*) to indicate the traditional, broad vs. the modern, restricted definitions.

The Atlantic Ocean

The Atlantic Ocean is the second largest of the world's oceans covering approximately 20% of the Earth's surface. The North Atlantic is the warmest and most saline of the world's oceans, having a mean potential temperature of 5.08°C and mean salinity of 35.09‰, compared to the global averages of 3.51°C and 34.72‰. The surface water temperatures range from less than 2° to 29°C. In the mid-latitudes, the area of maximum temperature variation, values may vary by 7° to 8°C over short time intervals (McCartney 1994).

An important feature of the Atlantic circulatory system (Fig. 1) is the cross-equatorial northward transport, by means of the South Equatorial Current (SEC), of surface waters that originated from the Indian Ocean and from the anticyclonic South Atlantic gyre.

Today, the surface circulation in the North Atlantic is dominated by the warm North Atlantic Drift (NAD) which is the northward extension of the Gulf Stream system (Dietrich et al. 1980). The NAD carries heat to the north and maintains the warm climates of central and northern Europe. After crossing the Mid-Atlantic Ridge near 50°N the NAD carries the major part of this water toward the north (Krauss 1986). Some of it enters the Norwegian Sea east of Iceland and some turns westward, flowing across the Reykjanes Ridge between 53°N and 60°N into the Irminger Sea. The northern boundary of the NAD is formed by the Subarctic Front that separates cold subpolar water from warmer Atlantic water (Krauss and Käse 1984).

The surface current system in the South Atlantic is dominated by a subtropical anticyclonic gyre and is closely coupled to lower atmospheric wind stress. In the eastern South Atlantic, the surface water circulation is dominated by the northward directed Benguela Current (BC), and the warmer southward flowing Angola Current (AC). The BC and AC converge between 14° and 16°S building a marked front (Angola-Benguela front) which is well defined in terms of both temperature and salinity. In addition, the prevailing winds in this region in turn drive an off-shore surface drift and cause coastal upwelling of cold, nutrient-rich water especially during austral winter. Upwelling occurs in a number of cells south of about 18°S with a major, in principle semi-permanent cell at 27°S (Shannon and Nelson 1996). The upwelling leads to enhanced biological productivity off Namibia. Further offshore the oceanic portion of the Benguela Current is characteristic for the upper-layer waters. This flow feeds into the broad, north-westward flowing South Equatorial Current (SEC), forming the eastern limb of the subtropical gyre (Fig. 1). The SEC itself consists of two branches, a mainstream flowing south of 10°S, and a trade wind-forced smaller, faster flowing branch between 2° and 4°S

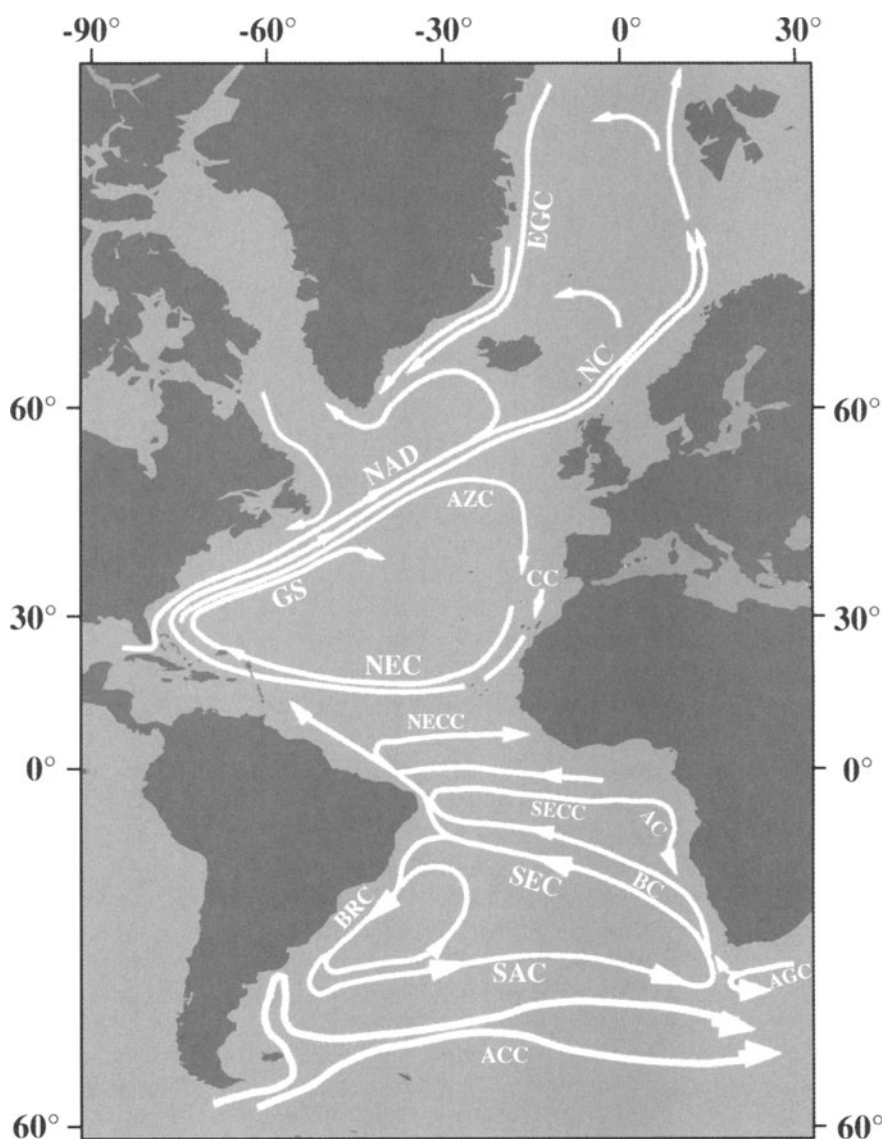


Fig. 1. Schematic map of the circulation in the upper levels of the Atlantic (redrawn from various sources). EGC = East Greenland Current, NC = Norwegian Current, NAD = North Atlantic Drift, AZC = Azores Current, GS = Gulf Stream, CC = Canary Current, NEC = North Equatorial Current, NECC = North Equatorial Counter Current, SEC = South Equatorial Current, SECC = South Equatorial Counter Current, AC = Angola Current, BRC = Brazil Current, BC = Benguela Current, SAC = South Atlantic Current, AGC = Agulhas Current, ACC = Antarctic Circumpolar Current.

(Peterson and Stramma 1991). In the equatorial area, these two branches are separated by the South Equatorial Counter Current (SECC), which moves surface water eastward. At about 10°S off Brazil the SEC splits into two branches, building the southward flowing Brazil Current (BRC) and the northward flowing North Brazil Current (Peterson and Stramma 1991). The latter contributes to the eastwards flowing North Equator Counter Current (NECC). Its interaction with the northern branch of the SEC leads to a strong convergence of water masses in the mixing area at about 3° to 5°N. This results in downwelling of surface waters, which supports the eastward flowing Equatorial Undercurrent.

At the southern border of the Subtropical Front located around 40°S, the warmer waters of the South Atlantic Current (SAC) parallel the cold and nutrient rich Antarctic Circumpolar Current (ACC) in the south. The boundary between subtropical and subantarctic water may extend regionally over a large area between 30° and 45°S (Smythe-Wright et al. 1998). Off the south-western tip of Africa the SAC meets the westward directed Agulhas Current (AGC) which consists of warm and saline Indian Ocean water. This causes most of the AGC to be retroflected back into the Indian Ocean, but also spinning off westward flowing rings, eddies and filaments which are responsible for heat and salt transfer into the South Atlantic.

Coccolithophorid biogeography – previous work

General coccolithophorid biogeography and habitat are relatively well-known from both surveys of the plankton and bottom sediments (Winter and Siesser 1994; Brand 1994; Roth 1994; Young 1994). With the exception of the investigation by McIntyre and Bé (1967) and Geitzenauer et al. (1977), most of the studies on recent sediments in the Atlantic Ocean are of rather small-scale, regional character (e.g. Gard 1987; Houghton 1988; Pujos 1988; Eide 1990; Giraudeau 1992; Baumann et al. 2000; Kinkel et al. 2000). In addition, multivariate statistical techniques to relate coccolith assemblages to physical and chemical parameters of surface water have only rarely been applied so far (Roth 1994).

Individual species typically occur in all the world's oceans but with more or less limited latitudinal distribution. Three to four broad coccolithophorid floral zones are recognized (McIntyre and Bé 1967; Okada and Honjo 1973; Winter et al. 1994): Subarctic, Temperate, Subtropical, Tropical and Subantarctic. These floral zones are consistently recognizable especially in oceanic plankton samples, and they are associated with major water masses. However, this is a simplistic distribution that doesn't take into account coastal currents, gyres, eddies, upwelling and rather obscures species specific biogeography. In addition, it doesn't show any difference between North and South Atlantic distribution. In the currently available coccolithophorid biogeographies the species assemblages are similar to their counterparts in the opposing hemisphere.

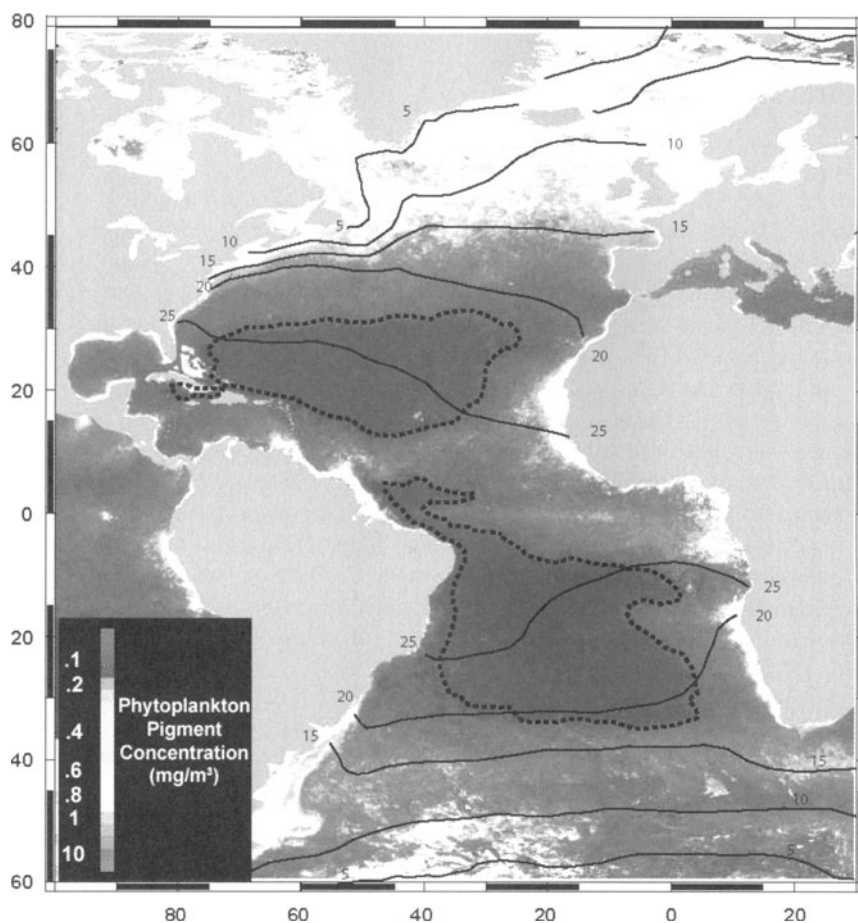


Fig. 2. Map of surface chlorophyll concentration from Nimbus-7 Coastal Zone Color Scanner data acquired between November 1978 and June 1986. Reproduced with permission of NASA, color original at http://seawifs.gsfc.nasa.gov/SEAWIFS/CZCS_DATA/global_full.html. The dark areas delineated by a dotted line correspond to the oligotrophic gyres, with mean chlorophyll concentrations $< 0.1 \text{ mg m}^{-3}$. The bright areas correspond to seasonally eutrophic regions, with mean chlorophyll concentrations $> 1 \text{ mg m}^{-3}$. Overlain are contours of mean annual surface water temperature, in degrees centigrade (World Ocean Atlas 2001 (Conkright et al. 2002)).

Methods

This study is based on surface sediment samples collected in the Atlantic Ocean from 86°N to 49°S. We eliminated samples that showed severe calcite dissolution following Geitzenauer et al. 1977. The water depth for the 490 samples ranged from 5427 to 187 m (average 3129 m). For details on water depth, position and authors, see Appendix 1 (see <http://www.coccoco.ethz.ch/ziveri>). In order to compare CLIMAP and new data we recalculated species abundances excluding *Florispheera profunda* (which was not included in the CLIMAP dataset).

The largest new dataset was obtained using the following preparation protocol. Between 40 and 100 mg of dry bulk sediment were suspended in tap water. The samples were ultrasonically treated for 30 seconds and then wet-split using an electrical rotary sample divider. One hundredth of the suspension was filtered through a polycarbonate membrane filter (47 mm diameter, 0.4 μ m pore size) by means of a low pressure vacuum pump. The filters were then oven-dried at 45°C for 24 hours. A randomly chosen filter section of around 1x1 cm was cut, fixed on an aluminum stub and sputtered with gold/palladium. Qualitative and quantitative examination of the coccoliths was carried out on a Scanning Electron Microscope (Zeiss DMS 940A) at magnifications of 3000 or 5000x.

The contour maps presented are obtained by using inverse distance to power gridding method, a weighted average interpolator. The program used is Surfer Version 6. For data analysis we have used graphical examination of the contour maps with reference to maps and overlays of mean annual temperature and annualized productivity, based on surface chlorophyll mapping (Fig. 2). This rather simple procedure was preferred to statistical analysis since we were primarily concerned with identifying the ecological preferences of individual species, rather than assemblage analysis. Moreover, since wide ecological contrasts occur in the area we would predict that most species have their distribution affected by both minima and maxima of environmental tolerance. In these circumstances correlation-based statistical methods are inappropriate. The South Atlantic dataset was previously analyzed and discussed by Böckel et al. (subm.).

Mean annual temperature was used since this is the simplest single measure of temperature and avoids the problems of comparing seasons between the hemispheres. Particularly at high latitudes, summer temperature might be considered a more significant measure, so we compared our data also with summer temperatures, but there were no obvious anomalies which were better explained by summer temperatures. Satellite derived surface chlorophyll was used rather than nutrient concentrations or other estimates of primary productivity since it provides a coherent dataset which showed obvious correlation with the coccolith patterns seen. There are known limitations to such data, in particular it is only a proxy for surface productivity. However, it remains an excellent way of distinguishing trophic regimes.

Description of species distribution

Coccolithus pelagicus (Fig. 3)

The species *C. pelagicus* produces the largest common coccoliths so it is very robust, essentially unmistakable, and well-documented. Nonetheless recent studies (Baumann et al. 2000; Geisen et al. 2002; Sáez et al. 2003; Geisen et al. this volume) have shown that extant *C. pelagicus* consists of at least two discrete sub-species, separable by morphology, molecular genetics and ecological preferences. Essentially there appears to be a sub-Arctic sub-species, *C. pelagicus pelagicus* producing coccoliths $<10\ \mu\text{m}$ long and a larger temperate sub-species, *C. pelagicus braarudii*, producing coccoliths $>10\ \mu\text{m}$ long.

Our biogeographic data (Fig. 3) primarily reflects the well-documented sub-Arctic sub-species, with a clear abundance maximum in the northern Atlantic, corresponding rather closely to areas with mean temperatures below 10°C (see also Samtleben et al. 1995). These are also areas of consistently high productivity (Fig. 2).

Outside this area the only occurrences of *C. pelagicus* registered in our dataset are in the Benguela upwelling area, off South West Africa, this population has been recorded by Giraudeau (1992) in sediment and by Giraudeau et al. (1993) in the plankton. Additional occurrences in temperate upwelling areas are, however, also recorded from off north-west Africa (Blasco et al. 1980) and on the Portuguese shelf (Cachao and Moita 2000), these occurrences are indicated by asterisks (Fig. 3). The Portuguese occurrence is registered in sediment assemblages (Cachao and Moita 2000), but is not sampled in our data. Obviously these populations are occurring at substantially warmer temperatures than the Arctic populations – the extant populations were recorded as occurring in waters of $15\text{--}18^\circ\text{C}$ in each area. This apparent anomaly was noted by Cachao and Moita (2000) but only resolved following recognition that the temperate upwelling populations constitute a separate sub-species. The two sub-species only co-occur in the central North Atlantic between about 35°N to 55°N .

The absence of a sub-Antarctic population of the Arctic sub-species, *C. pelagicus pelagicus* equivalent to that in the Arctic cannot be inferred from the data presented, since there are no samples from far enough south (most sediments in this area are non-calcareous). However, plankton studies have repeatedly shown that *C. pelagicus* is in fact absent from southern high latitudes in the Atlantic at the present day and in Holocene sediments (e.g. Verbeek 1989; Findlay and Giraudeau 2001), although it is present in Quaternary sediments (e.g. Flores et al. 1999). There are likely records of this form from cool waters around New Zealand and Australia (Nishida 1979; Hallegraeff 1984).

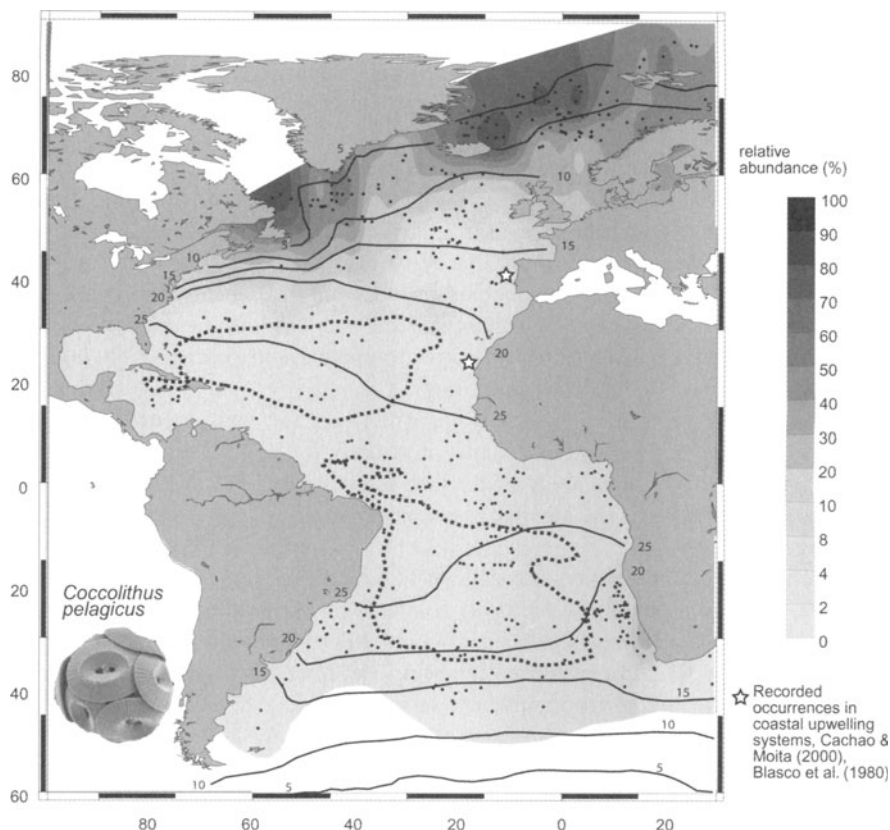


Fig. 3. Relative abundances of *C. pelagicus* (excluding *F. profunda*) in Atlantic Ocean surface sediments. Our biogeographic data primarily reflects the well-documented sub-Arctic *C. pelagicus pelagicus* with clear abundance maxima in the northern Atlantic, corresponding rather closely to areas with mean temperatures below 10°C. These are also areas of consistently high productivity (Fig. 2). Outside this area the only occurrences registered in our dataset are *C. pelagicus braarudii* in the Benguela upwelling area, off SW Africa, and in temperate upwelling areas off NW Africa and on the Portuguese shelf, these occurrences are indicated by asterisks. Solid line = annual mean sea surface temperature. Dotted line = oligotrophic gyre centers.

***Calcidiscus leptoporus* (Fig. 4)**

C. leptoporus is a well-established, diagenetically robust, globally distributed species. As discussed by Quinn et al. (this volume) it has been shown to consist of three sub-taxa, variously described as large, intermediate and small morphotypes (Knappertsbusch et al. 1997) or as separate sub-species (Geisen et al. 2002) or species (Sáez et al. 2003). Present knowledge suggests that the intermediate form

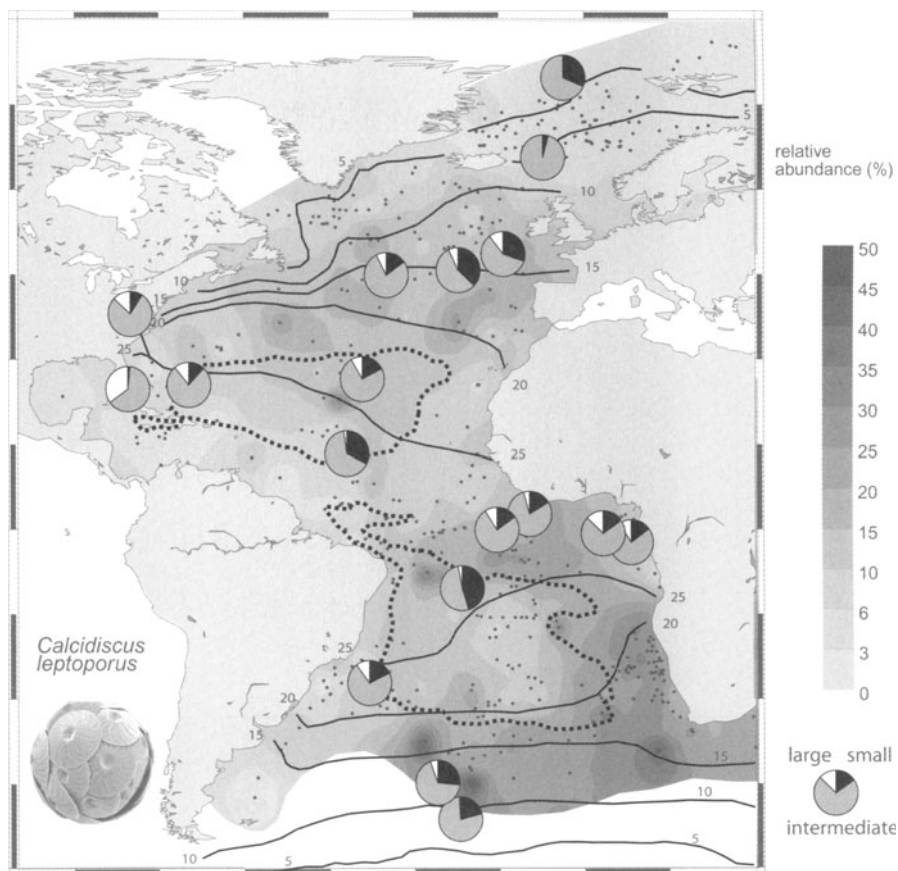


Fig. 4. Relative abundances of *C. leptoporus* (excluding *F. profunda*) in Atlantic Ocean surface sediments. The distribution broadly follows the counter-clockwise circulation around the central gyre and there is a clear abundance minimum in the oligotrophic gyre center (indicated by the dotted line). Data from 18 samples of morphotype distribution in the surface sediments by Knappertsbusch et al. (1997) is plotted. Solid line = annual mean sea surface temperature. Dotted line = oligotrophic gyre center.

has very wide distribution but with an affinity for cool, nutrient poor waters, whilst the large form occurs primarily in higher productivity, mesotrophic, environments (Renaud et al. 2002). The ecology of the small form is unclear, since it seems to have a very patchy distribution.

The mapped distribution (Fig. 4) shows a rather complex distribution with a marked contrast between the North and South Atlantic. In the South Atlantic abundances peaks are at high latitudes, approximately corresponding to the sub-Antarctic polar front and extending north along the Benguela eastern upwelling. These abundances broadly correspond to areas with a mean annual temperature below 20°C. High abundances are also seen in substantially warmer waters along

the southern edge of the mid-Atlantic divergence. Thus the distribution broadly follows the counter-clockwise circulation around the central gyre and there is a clear abundance minimum in the oligotrophic gyre center (indicated by the dotted line on Fig. 4). By contrast in the N. Atlantic abundances are lower and patchy. The central oligotrophic gyre is again a low abundance region but the temperate, higher abundance region to the north is only weakly developed.

It would be tempting to interpret the data as reflecting a combined distribution of different species or sub-species with discrete cool and warm water mesotrophic forms. However, available data on the morphotype distribution in the sediments does not support this. Data from 18 samples of Knappertsbusch et al. (1997) is plotted in Fig. 4 and additional data from the South Atlantic in Fig. 5. There is no very obvious relationship of the morphotype distribution to the pattern outlined above. The intermediate form dominates all samples which seems to indicate a broad ecological tolerance of this type. The large form tends to be more abundant in equatorial samples and is absent at higher latitudes, approximately coinciding with the 15°C mean annual temperature isotherm.

The study of Böckel et al. (subm.) showed that in the South Atlantic *C. leptoporus* is mostly encountered in the temperate to sub-polar regions of more eutrophic conditions. The positive correlation to elevated nutrient availability might be

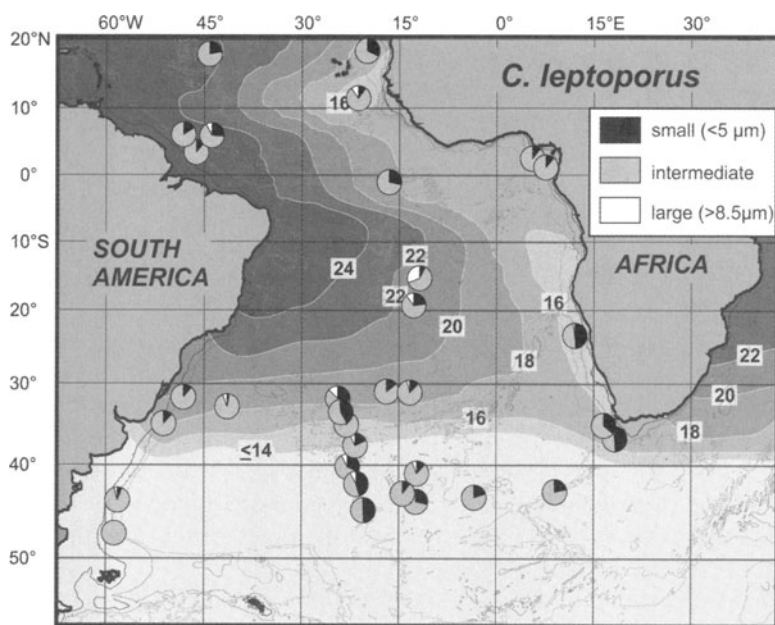


Fig. 5. Proportions of *C. leptoporus* morphotypes in South Atlantic samples analyzed by Böckel et al. (subm.). Shading = annual mean sea surface temperature ranges.

a primary response of *C. leptoporus*. This is in agreement with previous studies by Fincham and Winter (1989) and Andruleit and Rogalla (2002) who showed that this species responded positively to an increase in nutrient supply.

***Gephyrocapsa* spp. (Fig. 6)**

This is a genus which shows high morphological variability at the present day and rapid evolutionary change in the past (Bollmann et al. 1998). As a result it has been intensively studied by paleontologists in order to produce a high-resolution biostratigraphic subdivision of the Quaternary. The modern *Gephyrocapsa* assemblage is dominated by three species, all of which occur abundantly in surface sediments; *G. oceanica*, *G. muelleriae* and *G. ericsonii*. Of these *G. muelleriae* has well-established affinities for cool surface waters (Winter et al. 1994) and occurs in high abundances, even as the dominant taxon, in sediments underlying cool surface water (Bollmann 1997; Findlay and Giraudeau 2002). *G. oceanica* shows a general affinity for warmer waters and a very clear affinity for elevated nutrient concentrations (Kleijne et al. 1989; Giraudeau et al. 1993; Ziveri and Thunell 2000; Broerse et al. 2000). The ecology of *G. ericsonii* is not well established.

Bollmann (1997) showed that *Gephyrocapsa* could be further subdivided and that the morphotypes were most probably genotypically distinct taxa with discrete ecologies. He subdivided *G. oceanica* into an equatorial form (GE) and a larger form (GL) associated with upwelling regions. Similarly he recognized a temperate cool water (GC), an oligotrophic warm water morphotype (GO) and an intermediate morphotype (GT) which would all be classified as *G. muelleriae* by most authors.

Given this complex set of morphotypes we might predict that the multi-species genus *Gephyrocapsa* would fail to show a very consistent biogeographic pattern and indeed it does not. There is a clear minimum in the South Atlantic gyre center and several maxima associated with upwelling cells off the African margin but otherwise the pattern is one of rather uniformly moderate abundances. A clearer pattern emerges from the distribution of morphotypes. The symbols in Fig. 6 indicate the dominant morphotype in *Gephyrocapsa* assemblages analyzed by Bollmann (1997). The equatorial (GE = smaller *G. oceanica*) and cold (GC = *G. muelleriae* with low angle bar) morphotypes are associated with abundance maxima at respectively equatorial and temperate latitudes. By contrast the large, oligotrophic, transitional and small morphotypes are all associated with areas of low total *Gephyrocapsa* abundance at intermediate latitudes.

Complimentary data from Böckel et al. (subm.) on the distribution of the conventionally recognized species in the South Atlantic is given in Fig. 7. That study also included analyses of the species distributions with respect to the environmental parameters of the overlying surface waters. *Gephyrocapsa muelleriae* shows consistently high abundances in temperate areas reflecting its affinity for cool, nutrient-rich environments (Fig. 7b). Abundance of this species exhibits an inverse relation to temperature and its northern occurrence limit approximately

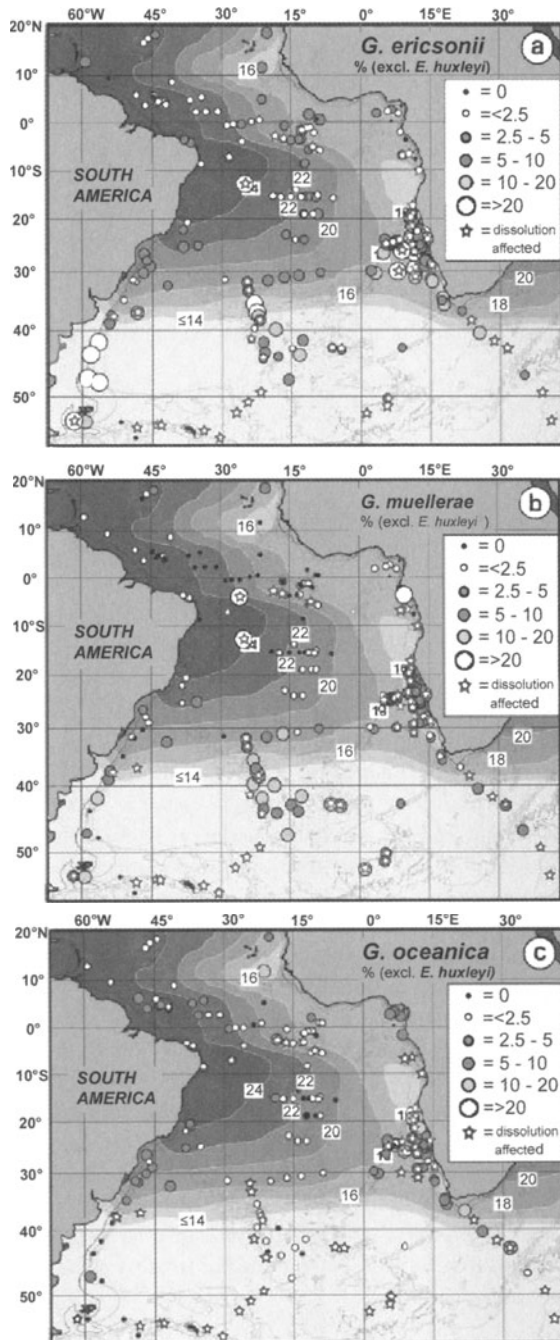


Fig. 7. Distribution maps of relative abundances (excluding *E. huxleyi*) of **a.** *G. ericsonii*; **b.** *G. muelleriae*; **c.** *G. oceanica* in the South Atlantic (Böckel et al. subm.).

abundances in the equatorial divergence, the Benguela upwelling zone and along the South American shelf. Statistical analysis showed a weak, but positive correlation with increased nutrient concentrations (Boeckel et al. subm.).

Gephyrocapsa ericsonii shows the broadest distribution but proved to be weakly correlated to cooler conditions, lower salinities, and to elevated nutrient concentrations (Boeckel et al. subm.). Thus, maxima in abundance of this species were observed along frontal systems with highly convoluted edges, such as the Benguela filamentous zone and the Subtropical Front (Fig. 7c). There are also notable high abundances on the South American shelf between 40° and 55°S, an area of high production and cool water. Its positive response to enhanced productivity conditions is confirmed by trap experiments from the Canary Islands region (Sprengel et al. 2002).

***Helicosphaera carteri* (s.l.) (Fig. 8)**

Most recent studies of extant coccolithophores have followed Jordan and Young (1990) and regarded the species as including three varieties (*carteri*, *wallichii*, and *hyalina*) separated on the basis of differences in the central area (see Geisen et al. this volume). New results from culture studies and molecular genetics, however, have suggested that these are three well-separated species (Sáez et al. 2003, Geisen et al. this volume). Probably they have discrete ecologies, but little data is available on their different biogeographies, although elevated abundances of *Helicosphaera carteri* var. *hyalina* coccospheres have been observed in the upwelling area off NW Africa (A. Kleijne pers. comm.; Ziveri et al. 2001).

Observations from plankton studies suggest that *H. carteri* has affinities for warmer water (McIntyre and Bé 1967; Brand 1994). In addition it shows an affinity for at least moderately elevated nutrient conditions as suggested by higher abundances in the mesotrophic parts of the San Pedro Basin (Ziveri et al. 1995a), the Arabian Sea (Andruleit and Rogalla 2002) and the Australian sector of the Southern Ocean (Findlay and Giraudeau 2002). In support of this, consistently low abundances of this species have been documented in the very oligotrophic (phosphate limited) waters of the eastern Mediterranean (Knappertsbusch 1993; Ziveri et al. 2000; Malinverno et al. in press).

The distribution map shows a remarkably strong pattern with a very clear maximum abundance in the mesotrophic eastern equatorial tropical divergence belt.

By contrast *H. carteri* is only present at low abundances or absent in the subtropical gyres. There is weak evidence of higher abundances in mesotrophic temperate waters but evidently it is excluded, probably by temperature, from high latitudes. The northern distribution margin approximately coincides with the 10°C mean annual temperature isotherm.

***Umbilicosphaera sibogae* (s.l.) (Fig. 9)**

As with most of the previously considered species *U. sibogae* is a convenient taxonomic entity which can be readily and reliably counted, by either light or electron microscopy. However, as with the other species recent research has proven, that it is made up of distinct sub-taxa. Two varieties have conventionally been distinguished, *U. sibogae* var. *sibogae* and *U. sibogae* var. *foliosa*. Molecular genetic data (Sáez et al. 2003) has shown that these are well-differentiated sister taxa that should be considered as separate species. They also have discrete life habits – *U. sibogae* var. *foliosa* is a typical placolith-bearing species forming compact coccospheres, whilst *U. sibogae* var. *sibogae* forms large colonial coccospheres containing 2–4 cells (see Geisen et al. this volume).

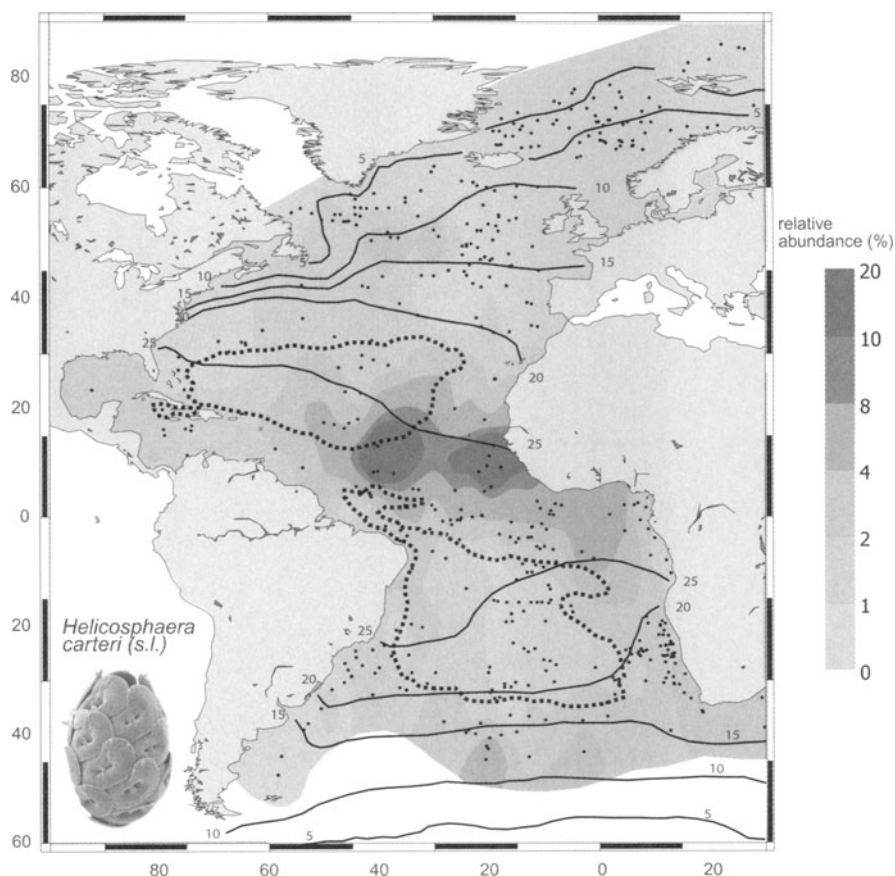


Fig. 8. Relative abundances of *H. carteri* (excluding *F. profunda*) in Atlantic Ocean surface sediments. Solid line = annual mean sea surface temperature. Dotted line = oligotrophic gyre center.

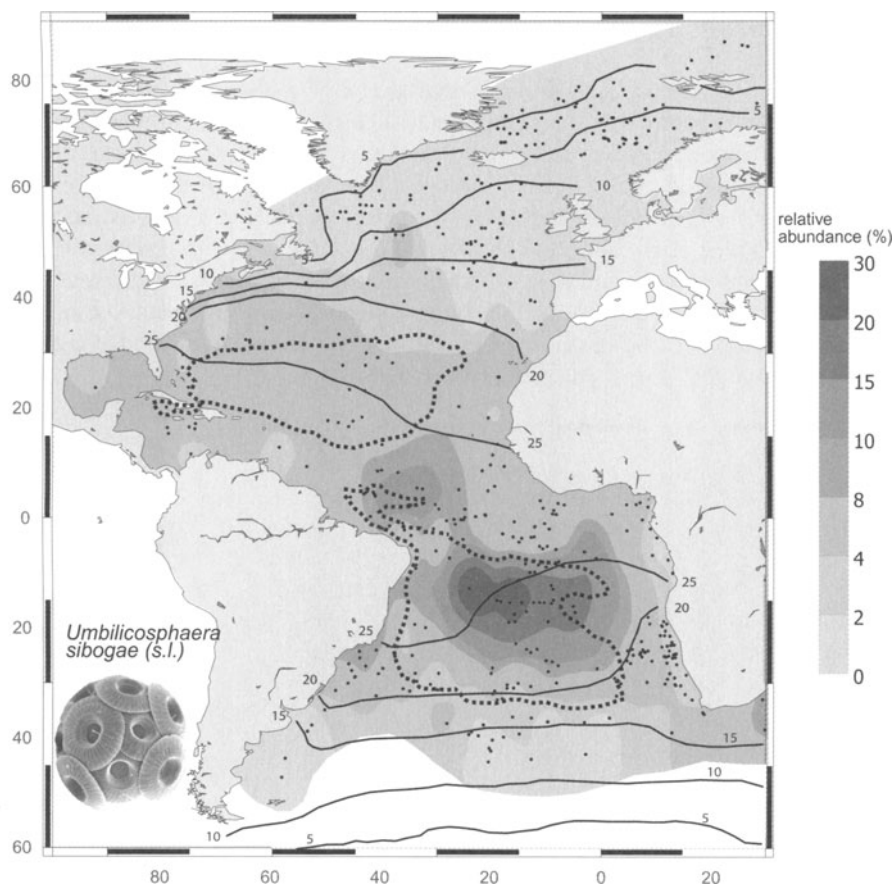


Fig. 9. Relative abundances of *U. sibogae*. (excluding *F. profunda*) in Atlantic Ocean surface sediments. Solid line = annual mean sea surface temperature. Dotted line = oligotrophic gyre center.

Statistical analyses of *Umbilicosphaera* abundance, (Böckel et al. subm.) revealed a weak preference for a warm, saline, slightly mixed upper waters of rather oligotrophic environments. Previous studies have suggested that *U. sibogae* var. *sibogae* mostly occurs under oligotrophic conditions whilst *U. sibogae* var. *foliosa* prefers mesotrophic conditions (Okada and McIntyre 1979; Ziveri et al. 1995b; Broerse et al. 2000; Ziveri and Thunell 2000). In most of the available datasets, however, the two varieties/species are not consistently separated so that a combined map is the only available option.

Despite the low taxonomic resolution, the distribution map (Fig. 9) shows a strong pattern. The species is clearly restricted to tropical and sub-tropical latitudes and to mean annual temperatures $>15^{\circ}\text{C}$. Moreover there is a well-defined abundance maximum in the South Atlantic corresponding rather closely to the

oligotrophic gyre, as defined by the surface water circulation and phytoplankton pigment concentration distribution (Fig. 2). Rather noticeably there is no equivalent abundance maximum in the North Atlantic. The South Atlantic abundance maximum of *U. sibogae* was also recorded by Lohmann (1919) and is evident in plankton data sets from the Atlantic Meridional traverse (A. Poulton pers. comm.), so it is not an artefact of the sediment record. Data from Boeckel et al. (subm.) show that the assemblages of the abundance maximum are dominated by *U. sibogae* var. *sibogae*. By contrast *U. foliosa* forms a significant part of the assemblages in the more mesotrophic areas. So the S. Atlantic abundance maximum is clearly a product of elevated abundances of *U. sibogae* var. *sibogae* strongly confirming its oligotrophic affinities.

***Syracosphaera pulchra* (Fig. 10)**

Syracosphaera pulchra is the largest extant *Syracosphaera* species and a useful representative of this diverse genus. Although there are numerous species in the genus, *S. pulchra* is morphologically distinctive and has been regarded as a well-defined species. However, recent results (Geisen et al. 2002 and this volume) have suggested that it comprises two species or sub-species, which are only readily distinguishable in the holococcolith bearing phase of their life-cycles. Holococcoliths are not well preserved in deep-sea sediments and consequently not systematically recorded in the datasets so all data is from the heterococcolith phase.

The map (Fig. 10) shows a rather widespread distribution up to about 50°N and S, with maximum abundances always below 8% (excluding *F. profunda*). Despite the low amplitude of the signal there is a distinct relative abundance maximum in the sub-tropical oligotrophic regions, rather loosely coinciding with the chlorophyll minima of satellite imagery (dotted line in Fig. 10). This is a clearer distribution than might have been expected since *S. pulchra* has not been regarded as one of the typical oligotrophic specialists. The S. Atlantic abundance maximum rather closely parallels that of *U. sibogae*, although at lower amplitude, but for this species there is an equally clear N. Atlantic abundance maximum. Conversely the equatorial abundance minimum almost exactly parallels the abundance maximum of *H. carteri*. It appears from this that *S. pulchra* at least in terms of broad sediment distribution is a relatively good indicator of sub-tropical oligotrophic waters.

An anomaly in the data is the weak occurrence maximum in the North Atlantic. This occurrence is under substantially more mesotrophic and cooler waters than the main sub-tropical occurrences. In this area Knappertsbusch and Brummer (1995) recorded a bloom of *S. pulchra* observed in sediment traps and as high abundances in plankton samples. Because this probably is a genuine occurrence, a possible explanation of this anomaly would be genetic differentiation between this temperate population and the main sub-tropical population.

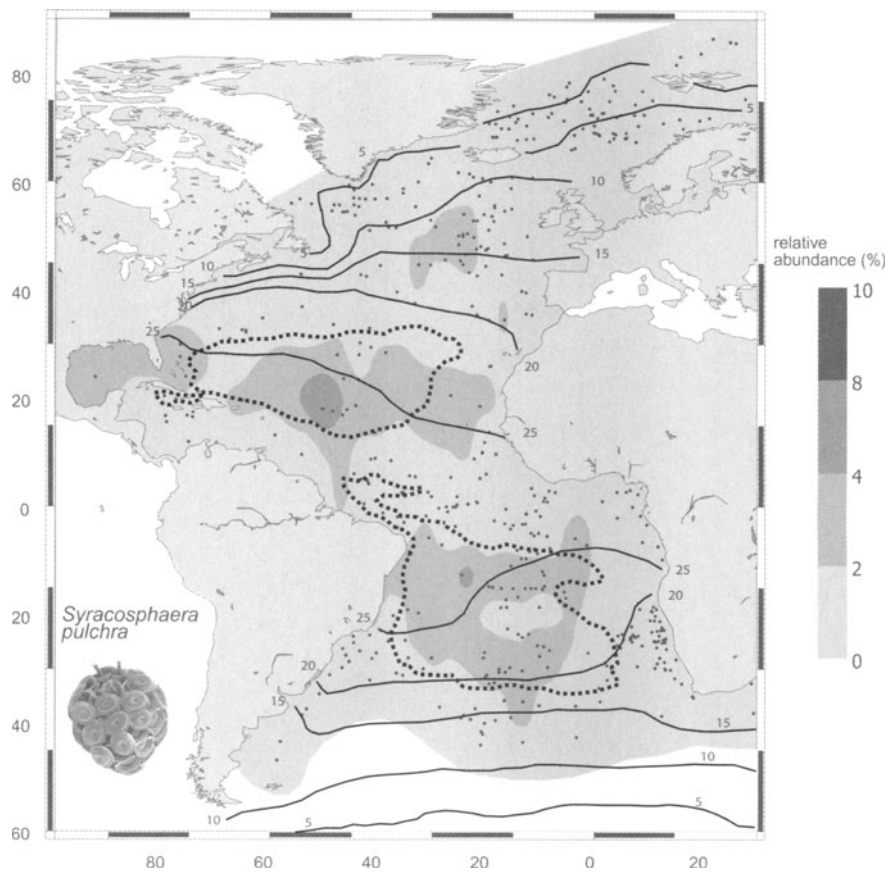


Fig. 10. Relative abundances of *S. pulchra* (excluding *F. profunda*) in Atlantic Ocean surface sediments. Solid line = annual mean sea surface temperature. Dotted line = oligotrophic gyre center.

Conclusions and outlook

As this study highlights, our knowledge of coccolithophorid biogeography and autecology is still limited. Although Atlantic coccolithophorid assemblages have been relatively intensively studied and a large dataset has been assembled here, there are manifest imperfections in it. Nonetheless, useful results can be inferred for each species and several general conclusions can be drawn.

Importance of fine-scale taxonomy

Perhaps the most obvious conclusion, suggested by virtually every case study, is that accurate taxonomy is essential for an understanding of ecology. Each of the taxa considered here represents an unambiguously defined morphological group, ideal for rapid low taxonomic resolution analysis of assemblages, which is a tempting strategy for ecological and paleoecological analysis of assemblages. However, in each case recent studies have indicated that these broadly defined taxa are in fact composed of several discrete species, or sub-species (see Geisen et al. this volume). It might have been expected, that such closely-related taxa would have similar ecological preferences. However, each of the case studies has shown that this is not the case, and that sub-taxa appear to have discrete ecological preferences and biogeographic distributions, even at the low geographic resolution achieved here.

The clearest example is perhaps *C. pelagicus*, with discrete sub-Arctic and temperate upwelling (sub-)species. For *Gephyrocapsa* and *Umbilicosphaera* the existence of subunits is less obvious but still unambiguous and is essential to understanding the biogeography of these taxa. Moreover in the case of *Gephyrocapsa* there is a strong suggestion that recognition of finer morphotypes, as described by Bollmann (1997), will allow better understanding of apparent biogeographic anomalies. For *H. carteri* and *S. pulchra* the mapped distributions are relatively straightforward and we do not yet know how they relate to the recently proven genotypic variation within the taxa. In both cases an obvious possibility is that less abundant outlier populations may prove to be dominated by different sub-taxa. Finally it has to be admitted that in the case of *C. leptoporus* the distribution pattern is still far from clear – however, high abundances are recorded in mesotrophic environments especially in the South Atlantic, but with an anomalously wide total temperature range. This species as a whole shows an implausibly broad ecological tolerance and subdivision into the now well proven morphotypes (or species, see Quinn et al. this volume) does not greatly clarify the distribution. This may indicate, as in *Gephyrocapsa* that a further level of genotypic diversity occurs in this species group or that more accurate assignment of specimens to the sub-taxa is needed.

The fact that even very closely related taxa have distinctly different ecologies and biogeographies has obvious implications for paleoecological analysis. On the positive side it is likely that for Late Quaternary and Holocene applications far better results can be expected from use of these insights. Conversely, if closely related taxa can have very different ecologies today, it obviously is unwise to extrapolate modern environmental preferences to older fossil assemblages.

Nature of biogeographic controls

As might be expected, most species show broad scale distribution patterns which can be related to the prime environmental parameters of temperature and trophic regime (see also Brand 1994; Winter et al. 1994; Young 1994). At high latitudes

temperature and productivity belts parallel each other and their effects are difficult to distinguish at least on this scale. At lower latitudes however, the effects are more clearly separable – it is for instance obvious that *S. pulchra* shows a warm water low productivity preference whilst *H. carteri* shows a warm water higher productivity distribution. Nonetheless some taxa show departures from a simple relationship to temperature and productivity which are difficult to explain in terms of imperfections in the dataset or inadequate taxonomy. In particular there are three cases where distribution patterns in the North and South Atlantic are distinctly different. These are the absence of *C. pelagicus* in the sub-Antarctic, the much higher abundance of *C. leptoporus* in temperate South Atlantic than North Atlantic, and the much higher abundance of *U. sibogae* var. *sibogae* in the oligotrophic South Atlantic than North Atlantic. The absence of *C. pelagicus* in the sub-Antarctic is well-known and could be due to many factors since there are major contrasts between the Arctic and Antarctic Oceans in terms of chemical, physical and biological oceanography. The *Calcidiscus* and *Umbilicosphaera* patterns are more ambiguous, since the North and South Atlantic show broadly similar habitats in terms of temperature, salinity, productivity and macronutrients (nitrate, phosphate and silicate). Possible alternative hypotheses to explain these anomalies are either that the populations in the two oceans are sufficiently separated to have evolved slightly different ecological tolerances or that an additional factor, such as a trace element is responsible for the distribution contrasts. We think that investigating the causes of these distribution anomalies could prove a highly informative focus for future research. More broadly we suggest that the widespread recognition of comparable, broad coccolithophorid biogeographic zones in all oceans and the absence of obvious vicariance in coccolith species distributions has lead to significant contrasts between oceans, which so far have been overlooked and that such contrasts may provide important clues for interpreting past temporal shifts in assemblage compositions.

Outlook

Clearly our knowledge of coccolithophorid biogeography is still very imperfect. However, it is clear that there is great potential for well-focused research in this area, particularly from using a combination of molecular genetic and morphological techniques to map fine-scale morphotypes and to determine their ecological preferences. Moreover critical re-evaluation of even low resolution data can reveal inter-oceanic contrasts in distribution which provides clues to fundamental controls on phytoplankton biogeography.

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Why is the Land Green and the Ocean Red?

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Summary

Fossil evidence suggests that during the Paleozoic Era, green algae dominated eukaryotic phytoplankton taxa. One branch of this originally aquatic clade colonized terrestrial ecosystems to form what would become a green hegemony on land – with few exceptions, terrestrial plants are green. In contrast to land plants, contemporary oceanic phytoplankton are represented by relatively few species that are phylogenetically deeply branching. Since the Triassic Period, the major taxa of eukaryotic phytoplankton preserved in the fossil record have been dominated by organisms containing plastids derived from the “red”, chlorophyll *c* containing algal clade. The ocean became “red” sometime during the Triassic or early Jurassic periods. The evolutionary success of the red line in Mesozoic and younger oceans appears related to changing oceanic conditions. In this chapter, we briefly explore the evolutionary processes and ecological traits that potentially led to the success of the red line in the oceans.

Introduction

All eukaryotic photosynthetic organisms are oxygenic (Falkowski and Raven 1997). The apparatus responsible for the photochemical production of oxygen is contained within distinct organelles, called plastids, that retain a complement of DNA, but are incapable of self-replication without supporting genes that are resident in the host cell's nuclear genome. Based on small subunit ribosomal RNA

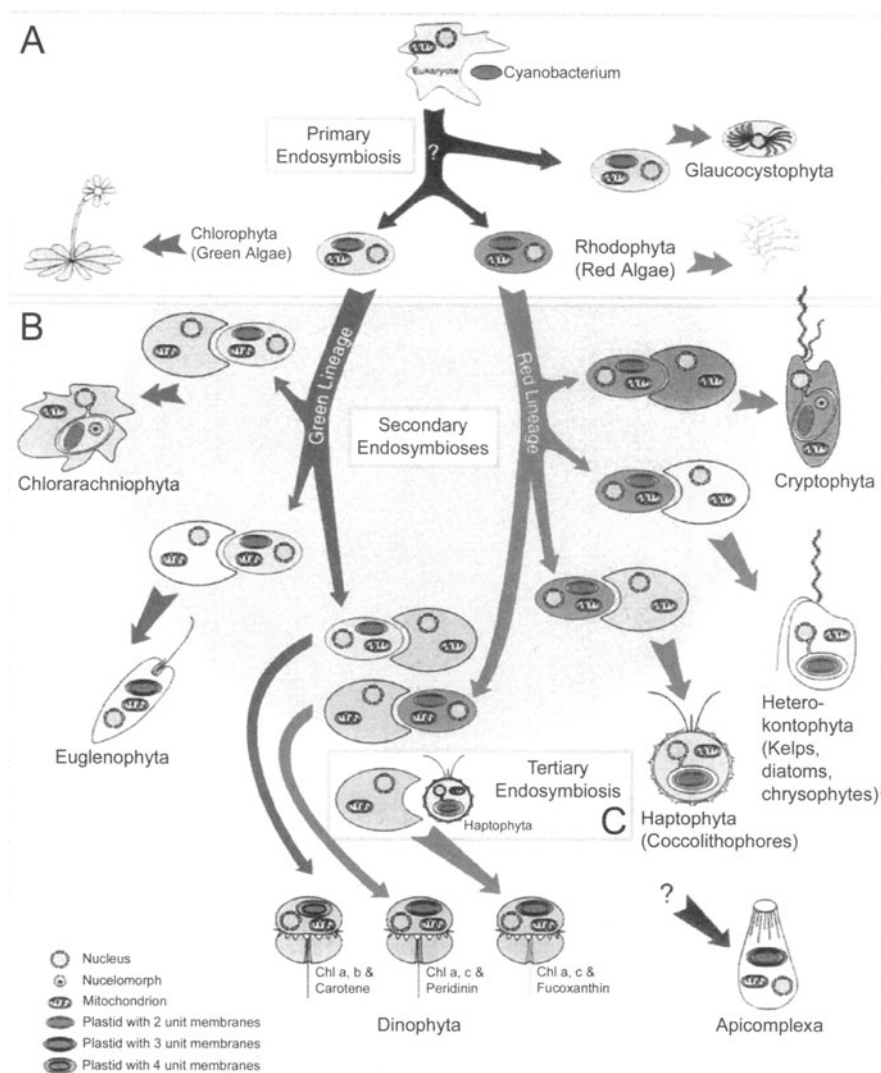


Fig. 1. The evolutionary inheritance of red and green plastids in eukaryotic algae. The ancestral eukaryotic host cell appropriated a cyanobacterium to form a primary photosynthetic symbiont. Three groups split from this primary association: one formed a “green” line, one a “red” line and the third is represented by the Glaucocystophyta. One member of the green line was the progenitor of higher plants. Two members of the extant green line form secondary eukaryotic associations, however neither of these taxa are ecologically significant. Several, independent secondary associations were formed from the primary red symbiont, including the haptophytes, heterokonts, and chrysophytes. The dinoflagellates appropriated plastids from both red and green lineages; however, the dominant group in the contemporary ocean is overwhelmingly red. (Adapted from Delwiche (1999), with permission).

sequences, it would appear that all plastids are derived from a single common ancestor that was closely related to extant cyanobacteria (Bhattacharya and Medlin 1995; Palmer 2003); however, early in the evolution of eukaryotic photoautotrophs major schisms occurred that gave rise to two major clades from which all eukaryotic photoautotrophs descended (Delwiche 1999) (Fig. 1). While all eukaryotic photoautotrophs contain chlorophyll *a* as a primary photosynthetic pigment, one group utilizes chlorophyll *c* and the other appropriated chlorophyll *b* as primary accessory pigments. No extant chloroplast contains all three pigments.

The chlorophyll *c* containing plastid lineage is widely distributed among at least six major groups (i.e., phyla or divisions) of aquatic photoautotrophs, but with the exception of some soil-dwelling diatoms and xanthophytes, is not present in any extant terrestrial photoautotroph. In contrast, the chlorophyll *b* containing plastid lineage is in three groups of eukaryotic aquatic photoautotrophs and in all terrestrial plants. Because additional accessory pigments (carotenoids) found in the chlorophyll *c* containing group have yellow, red, and orange reflectance spectra (i.e., they absorb blue and green light), the ensemble of organisms in this group are referred to, in the vernacular, as the “red lineage”, most of which are members of the sub-Kingdom Chromista (Table 1). The chlorophyll *b* containing group contains a much more limited set of carotenoids in the chloroplast, and members of this group generally have a green color. Thus, in effect, the ensemble of organisms responsible for primary production on land is green, while the ecologically dominant groups of eukaryotic photoautotrophs in the contemporary oceans are red. In this chapter, we explore the fossil evidence of the radiations of eukaryotic phytoplankton and consider some hypotheses that may account for the origin and ecological success of the red line in the oceans, while the green line maintained genetic hegemony on land.

Phytoplankton evolution in the geologic record

Long-term, macroevolutionary trends

Although the fossil record is clearly incomplete, analysis of microfossils from the Proterozoic through the end-Permian suggests that early oceans were not always dominated by the red lineage. For most of our planet’s Proterozoic history, cyanobacteria were probably the primary producers in the oceans, with eukaryotic algae rising to taxonomic and ecological prominence only near the end of the eon (Knoll 1992; Anbar and Knoll 2002). Well preserved fossils of vaucherian algae show that the secondary endosymbiosis that initiated “red lineage” diversification had already taken place by the Neoproterozoic (German 1990; Butterfield 2000). However, fossils in Neoproterozoic and especially Paleozoic shales indicate that phytoplankton with morphological features similar to members of extant green lineages were abundant and diverse in waters overlying contemporary continental shelves (e.g., Tappan 1980). For example, the prasinophyte, *Tasmanites* spp. (Fig.

Table 1. The higher taxa of oxygenic photoautotrophs, with estimates of the approximate number of total known species, and their distributions between marine and freshwater habitats*

Taxonomic Group	Known Species	Marine	Freshwater
Empire: Bacteria (= Prokaryota)			
Kingdom: Eubacteria			
Subdivision: Cyanobacteria (sensu strictu) (= Cyano- phytes, blue-green algae)	1,500	150	1,350
Subdivision: Chloroxybacteria (= Prochlorophyceae)	3	2	1
Empire: Eukaryota			
Kingdom: Protozoa			
Division: Euglenophyta	1,050	30	1,020
Class: Euglenophyceae			
Division: Dinophyta (Dinoflagellates)			
Class: Donophyceae	2,000	1,800	200
Kingdom: Plantae			
Subkingdom: Biliphyta			
Division: Glaucocystophyta			
Class: Glaucocystophyceae	13	—	—
Division: Rhodophyta			
Class: Rhodophyceae	6,000	5,880	120
Subkingdom: Viridiplantae			
Division: Chlorophyta			
Class: Chlorophyceae	2,500	100	2,400
Prasinophyceae	120	100	20
Ulvophyceae	1,100	1,000	100
Charophyceae	12,500	100	12,400
Division: Bryophyta (mosses, liverworts)	22,000	—	1,000
Division: Lycopsidea	1,228	—	70
Division: Filicopsida (ferns)	8,400	—	94
Division: Magnoliophyta (flowering plants)	(240,000)	—	—
Subdivision: Monocotyledoneae	52,000	55	455
Subdivision: Dicotyledoneae	188,000	—	391
Kingdom: Chromista			
Subkingdom: Chlorenchia			
Division: Chlorarachniophyta			
Class: Chlorarachniophyceae	3–4	3–4	0
Subkingdom: Euchromista			
Division: Cryptophyta			
Class: Cryptophyceae	200	100	100
Division: Haptophyta			
Class: Prymnesiophyceae	500	100	400
Division: Heterokonta			
Class: Bacillariophyceae (diatoms)	10,000	5,000	5,000
Chrysophyceae	1,000	800	200
Eustigmatophyceae	12	6	6
Fucophyceae (brown algae)	1,500	1,497	3
Raphidophyceae	27	10	17
Synurophyceae	250	—	250
Tribophyceae (Xanthophyceae)	600	50	500
Kingdom: Fungi			
Division: Ascomycotina (lichens)	13,000	15	20

*The difference between the number of marine and freshwater species, and that of known species, is accounted for by terrestrial organisms. Dashes indicate that no species are known (by us) for their particular group in this environment.

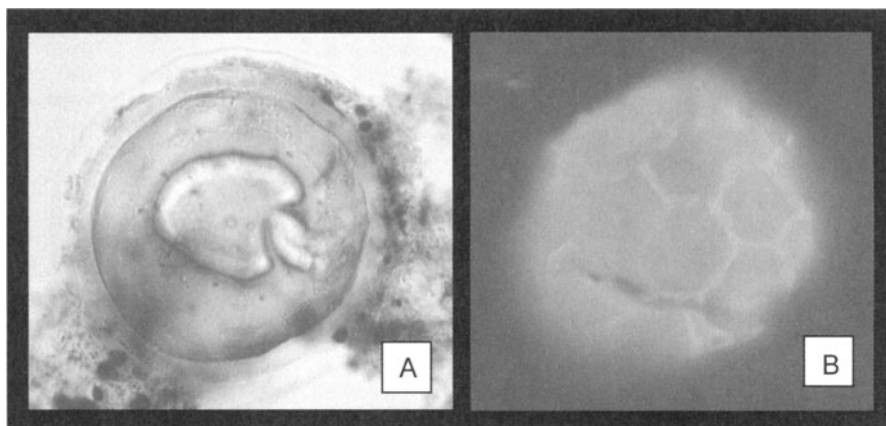


Fig. 2. The association of prasinophytes with anoxic conditions is also evident in the Early Jurassic (Toarcian) Posidonia Shales of NW Europe that may contain up to 105 prasinophyte cysts/g rock (Feist-Burkhardt 1992; Prauss et al. 1989; Prauss et al. 1991). However, the Toarcian appears to have been the last time these so-called “disaster species” (Tappan 1980) dominated organic-rich sediments on a global scale. Marine prasinophytes declined in importance during the Jurassic and Cretaceous and do not seem to have been major constituents of later Mesozoic ocean anoxic events (OAEs). Extant relatives of *Tasmanites* (e.g., *Halosphaera viridis*, *Pachysphaera* spp.) occasionally bloom in Norwegian and North Sea coastal waters, in the sub-arctic Pacific, as well as in the Mediterranean during winter and early spring blooms, however they seldom dominate eukaryotic phytoplankton assemblages (Van de Schootenbrugge et al. in press).

2) and other prasinophyte phycomata (e.g., *Cymatiosphaera*, *Dictyotidium*) are relatively common in Paleozoic black shales. Tyson (1995) suggested the abundance of these cells was “more than a preservational effect”.

In the Middle Triassic, two new groups of single-celled photosynthetic algae emerged in the fossil record (Fig. 3). One, the dinoflagellates, is recorded by carbonaceous microfossils with distinctive walls of polymerized glycans, relict cysts that were preserved in shallow marine continental margin regions. While dinoflagellates may be present among Neoproterozoic and Paleozoic microfossils (e.g., Moldowan and Talyzina 1998), identification remains uncertain. Further, although molecular biomarkers of dinoflagellates, such as the sterane and dinosterane, are present in Neoproterozoic sedimentary rocks, these markers only become prominent constituents of marine bitumens in the Triassic, when microfossils more securely document their radiation (Moldowan et al. 1996). The second group to radiate was the coccolithophores, a clade within the haptophytes that is armored with miniature plates (holococcoliths) of calcite (see Young et al. this volume). Later in the Mesozoic Era, the silica-encased diatoms emerged. Although Rothpletz (1896) reported diatom frustules in Jurassic sediments, the observations have proven difficult to replicate by later workers. Unquestionably,

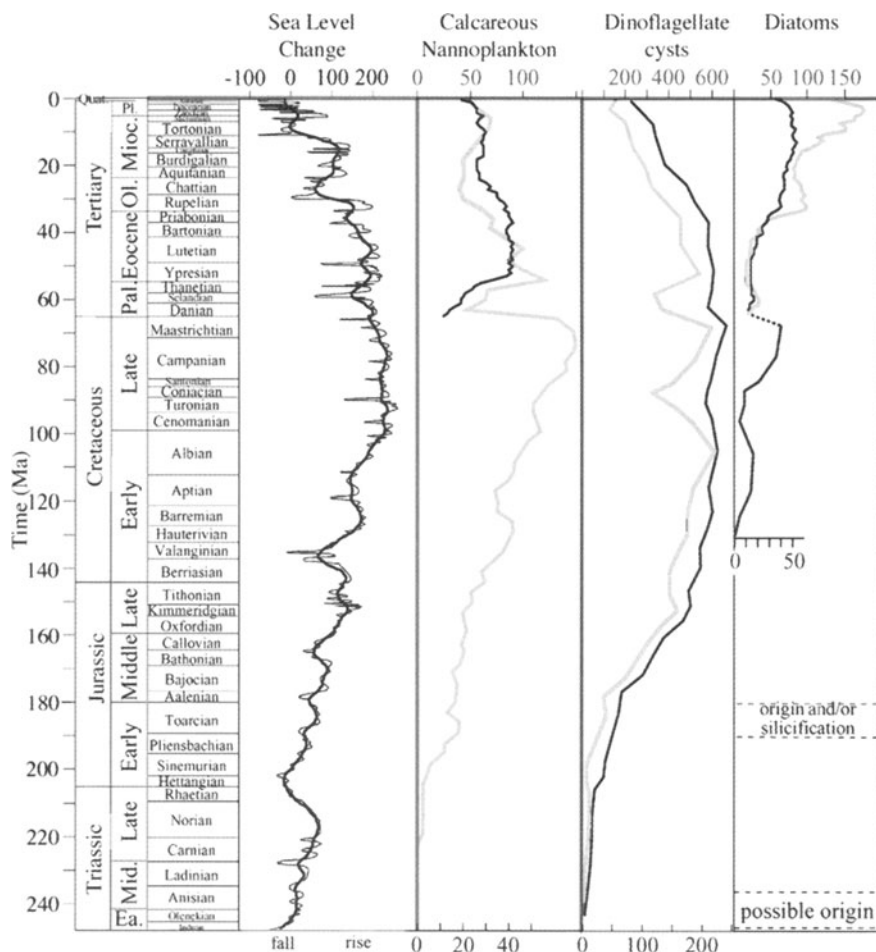


Fig. 3. Eukaryotic phytoplankton diversity curves compared with sea-level change. Phytoplankton species diversities (gray) are from published studies (calcareous nannofossils, Bown et al. this volume; dinoflagellates, Stover et al. 1996; diatoms, Spencer-Cervato 1999). Phytoplankton genus diversities (black) were compiled from publicly available databases (calcareous nannofossils and diatoms, Spencer-Cervato 1999; dinoflagellates, Mac Rae unpublished data). Sea-level curve is from Haq et al. (1987). All records are adjusted to a uniform time-scale (Berggren et al. 1995 for the Cenozoic and Gradstein et al. 1995 for the Mesozoic). (After Katz et al. in review)

however, diatoms had begun to radiate in the oceans by the Early Cretaceous (Harwood and Nikolaev 1995) and were present in non-marine environments by 70 million years ago (Chacon-Baca et al. 2002).

All three groups, the dinoflagellates, the coccolithophores, and the diatoms and their relatives, contain red plastids. These groups came to largely, but not totally displace green eukaryotic algae from Mesozoic time to the present.

Mesozoic-Cenozoic Phytoplankton Evolution

In spite of continued controversy over the details of evolutionary origins, the fossil record suggests that diversity within the red phytoplankton lineage was low in the early Triassic and increased rapidly in the Early Jurassic (e.g., Tappan and Loeblich 1988; Harwood and Nikolaev 1995; Stover et al. 1996; Bown et al. this volume). The long-term radiations in the Jurassic coincide with the opening of the Atlantic Ocean, accompanied by a sustained increase in sea level that flooded continental shelves. Indeed, observed Mesozoic increases in diversity of eukaryotic phytoplankton parallel the rise in sea level that began in the Early Jurassic (Haq et al. 1987; Hallam 2001) (Fig. 3).

In part, this correspondence surely reflects preservation and sampling – sediment abundance and availability commonly correlate with apparent diversity. Nonetheless, insofar as the Mesozoic diversity increase is apparent both within and among assemblages, biological explanations must contribute to observed patterns of fossil diversity. We suggest that the correspondence between sea stand and the radiation of the red lineages in the Jurassic may be related to life-cycle strategies. All three red eukaryotic taxa, the dinoflagellates, coccolithophores, and diatoms, produce resting stages; that is, following a bloom, a small fraction of the population of cells becomes arrested in a specific portion of a life cycle, increases the production of cell armor, and sinks to the sediments until such time as it is favorable to bloom (usually the following year). The timing of the bloom is generally keyed to changes in day length and water temperature. The resting stage is often associated with gamete formation and gene exchange in the planktic portion of the life cycle. This life cycle strategy for photoautotrophic organisms requires relatively shallow ocean regions, but moreover, promotes genetic isolation by reducing the planktic life cycle to well defined periods. Gene transfer, through sexual recombination and/or lateral vectors (such as viral infection), is highly attenuated in benthic stages. The genetic isolation may, over relatively short periods of time, have increased the tempo of evolution (i.e., rate of speciation) and phenotypic selection; processes that are observable in contemporary phytoplankton assemblages (Medlin et al. 1997).

Phytoplankton diversity was transiently uncoupled from long-term sea-level trends at the Cretaceous-Tertiary boundary, when mass extinctions, caused by a bolide impact, removed much of the diversity that had developed over the preceding hundred million years, especially among coccolithophores and dinoflagellates. Although dinoflagellate and, to some extent, coccolithophorid diversities recovered by the earliest Eocene, they began a long-term decline in the mid-Eocene; a trend that continues to the present time. Among fossilizable taxa, modern dinoflagellate species diversity is similar to levels found in the early Middle Jurassic, whereas modern coccolithophorid species diversity has dropped to levels last seen in the Late Jurassic. The decline of species richness in both groups corre-

sponds with a long-term regression in sea level, primarily associated with the resurgence of permanent polar ice at the beginning of the Oligocene. In contrast to the dinoflagellates and coccolithophores, however, the tempo of evolution of diatoms has been extraordinary from the Eocene/Oligocene boundary to the present.

Rise of the red lineages and competition among them

It is certainly possible that the radiations of diatoms, coccolithophores, and dinoflagellates reflect taxon-specific adaptations without a common explanatory basis. For example, it could be argued that diatoms expanded because of ecological advantages such as the retardation of predation or viral attack conferred by the evolution of the organosilicate frustules (Smetacek 1999; Hamm et al. 2003). Alternatively, we might seek explanations in the common features of their metabolism or in secular, long-term changes in marine environments. There are several issues embedded in the macroevolutionary pattern of eukaryotic phytoplankton, however, that are not readily explained by or correlated with such physical drivers. We can cluster these issues into three fundamental questions:

1. Why did the green lineage rapidly decline in ecological importance during the early Mesozoic? Like the “red” groups, prasinophyte green algae have resting stages that, in principle, could have fueled diversification as sea level rose in the Mesozoic. However, this did not happen. Moreover, major sea-level rise accompanied the Cambro-Ordovician radiation of eukaryotic phytoplankton, but did not foster a radiation of red lineage taxa. Thus, to explain why the red lineage rose to prominence in the Mesozoic, we must ask how the Mesozoic oceans differed from that of the Early Paleozoic.
2. What circumstances facilitated the radiation of the dinoflagellates and coccolithophores, but not the diatoms, in the Mesozoic, and why have the two former groups receded, while the last has risen to prominence in the Cenozoic?
3. Why have terrestrial photoautotrophs not followed similar trajectories following the end-Permian extinction? Or, simply put, why has the land remained green while the ocean became red?

We consider these issues in turn.

Selective pressures in Mesozoic oceans

Light

One potential selection mechanism for red and green plastids is spectral irradiance. Compared to land plants, the majority of the phytoplankton biomass in the oceans is light-limited for growth and photosynthesis. On land, competition for light within a canopy is based on total irradiance, not primarily on the spectral

Trace elements and ocean redox chemistry

The end-Permian extinction appears to be marked by hypoxic, if not anoxic oceanic conditions (Isozaki 1997). Such conditions alter the distributions of many trace elements (Whitfield 2001), thereby potentially exerting selective pressure on specific taxa of phytoplankton. Indeed, elemental analysis of 16 species of marine phytoplankton from six taxonomic groups reveals clear, taxon-specific patterns of variation in chemical composition (Fig. 5), that largely cluster, with overlaps, among red, green and cyanobacterial lineages (Quigg et al. 2003). The primary differences between taxa appear to correlate with the differential distributions of trace elements. For example, algae with green plastids have 3–4 fold higher quotas for iron and copper (i.e., Fe:P and Cu:P ratios) than red eukaryotes, while the latter have higher quotas for Cd, Co and Mn. Based on pairwise t-tests and ANOVA, the differences are statistically different. Clearly these differences in trace element composition must reflect phenotypic expression that is ultimately related to a fundamental genomic schism between the green and red lineages.

The genes responsible for the distribution of these trace elements are not localized in the plastid, yet the cells segregate along clade-specific lines according to

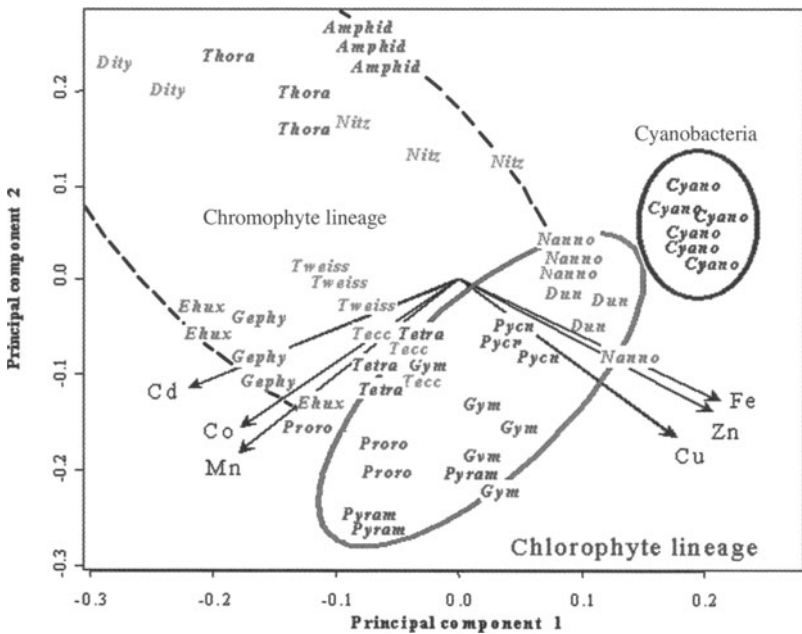


Fig. 5. A principal component analysis of the elemental composition of 16 species of algae including cyanobacteria, and eukaryotic red and eukaryotic greens. The results suggest that trace elemental composition can separate these major groups. (Adapted from Quigg et al, 2003.)

plastid type. This apparent paradox can be reconciled by recognizing that upon acquisition of either a primary or secondary plastid, the host cell appropriates and retains numerous plastid-encoded genes in the nucleus. The gene transfers appear to occur early in the evolutionary history of the symbiotic association (Delwiche 1999; Grzebyk et al. 2003). Hence, the phenotypic manifestation of trace element composition reflects selection pressures that occurred early in the evolution of the major phytoplankton taxa, and predicts a highly conserved set of primary and secondary gene products that are specific to a plastid inherited superfamily.

The elemental composition of dinoflagellates provides one test of this “plastid imprint” hypothesis. Dinoflagellates are promiscuous; they appropriate plastids from several primary and possibly secondary symbionts (Fig. 1). Assuming that the genetic backgrounds of the primordial host cells were similar, the plastid imprint hypothesis would predict that the trace element composition of dinoflagellates with plastids inherited from the red lineage would cluster more closely with other members of the red superfamily, while dinoflagellates with green plastids would cluster with the green superfamily. Indeed, based on trace element profiles, the three chlorophyll *c* containing dinoflagellates we examined, *Thoracosphaera heimii*, *Prorocentrum minimum* and *Amphidinium carterae*, are affiliated with the red lineage, and the chlorophyll *b* containing dinoflagellate, *Gymnodinium chlorophorum*, is associated with the green lineage. However, the host cells’ genetic backgrounds of these four species do not discriminate among the plastid types based on C:N:P stoichiometry (Quigg et al. 2003). These results strongly imply that plastid inheritance, but not contemporary plastid genomes, is a key factor dictating the trace element quotas in marine phytoplankton.

These results lead to the hypothesis that the phenotypic memory of trace element composition of eukaryotic phytoplankton is related to redox chemistry of the ocean. These biologically important trace elements are redox sensitive. Thus, their abundances in seawater through time will reflect the oxidation state of the oceans but may or may not be altered with diagenesis. For the first half of recorded Earth history, pO_2 appears to have been low in both surface and deep water masses (e.g., Holland 1984). Under these circumstances, Fe and Mn would have been abundant (relative to the requirements of photoautotrophs), whereas Cu and Mo would have been scarce (Williams 1981; Lipps 1993; Falkowski and Raven 1997; Raven 1997; Whitfield 2001; Anbar and Knoll 2002). Surface waters became oxic early in the Proterozoic Eon, yet the persistence of banded iron formations for more than six hundred million years after the initial rise of oxygen indicates that oxygen tensions must have remained low in many subsurface water masses (Canfield 1998). Indeed, black shales deposited beneath an anoxic water column are relatively common in Proterozoic marine successions (Shen et al. 2002; Shen et al. 2003), and widespread black shales persist through much of the Paleozoic Era (Arthur and Sageman 1994).

Deep-water anoxia may have been particularly pronounced near the end of the Permian Period (Isozaki 1997), and whatever events caused end-Permian mass extinction also introduced a remarkable, expanded oxygen minimum zone that persisted through the Early Triassic (Twitchett 1999; Wignall and Twitchett 2002). Widespread anoxic events occurred in the Mesozoic oceans, but were suf-

ficiently infrequent such that each draws widespread notice from geologists (e.g., (Arthur and Sageman 1994). In the Cenozoic record, global oceanic anoxic events (OAE) are essentially absent.

From the foregoing comments, one can conclude that temporal changes in the availability of trace metals broadly coincide with the biological transition to red lineage dominance of phytoplankton. Thus, long-term changes in the ventilation of the world's oceans may have been significant in turning the seas red during the Mesozoic. The causes of changes in redox chemistry remain obscure; however, OAEs are recorded in the Triassic, at the Triassic-Jurassic boundary and several times later in the Mesozoic. While ocean anoxia leads to increased availability of Fe, Co, P and simultaneously to decreased Cu, Zn, and Cd, the return to oxic conditions would have witnessed a reversal in the availability of redox sensitive elements (Williams 1981; Raven 1997; Whitfield 2001). However, once established, members of the red lineage retained their ecological advantage as eukaryotic photoautotrophs. Hence, the initial selection pressure in oceanic ecosystems (i.e., redox state) may have permitted ecosystem displacement of the green lineage by the red lineage, but, in and of itself, this phenomenon cannot explain the continued success of the red lineage for the past 250 Ma.

We note that such redox based selection pressure almost certainly was not a factor in terrestrial plant evolution; throughout all of the Phanerozoic, atmospheric concentrations of oxygen have been well above any threshold to alter trace element chemistry in soils or precipitation. The dramatic changes in ocean redox chemistry were not mirrored in terrestrial ecosystems after the rise of the eukaryotic algae. Thus, there was no selection against green photoautotrophs and their high metal quotas. In conjunction with biochemical traits that enabled greens to thrive in the harsh conditions inherent to the land surface (Knoll et al. 1986), this ensured that while the oceans turned red, the continents bloomed green. The absence of this strong selective force allowed the green photoautotrophs, which had already established a foothold on land, to diversify through their morphology to better cope with the major stresses encountered on land.

Plastid portability

The continued success of the red lineage throughout the Mesozoic to present time requires one or more processes or properties that maintains evolutionary competitive advantage under a wide range of climatological, physical, and chemical conditions. One potential property that differentiates between the evolutionary trends and ecological success of the red and green lineage is contained in the plastid genome. Following an endosymbiotic event, most of the genes in the photosynthetic partner were lost or transferred to the host cell nucleus (McFadden 1999). Could there be a differential segregation of genes between red and green plastids that accounts for the success of the former in a widely diverse set of host cells?

Fig. 6. Distribution of the 190 first identified and 66 hypothetical (ycf) protein-coding genes in nine photosynthetic algal plastid genomes. Primary endosymbiotic genomes (Glaucoophyta, Rhodophyta, Chlorophyta) are represented with square boxes, and the secondary endosymbiotic genomes (Euglenophyta, Cryptophyta, Bacillariophyta) with circles. Genes involved in the three main plastid functions represented in the core set are highlighted: ATP synthase genes (—), photosynthetic processes (·····), and housekeeping genes (· · ·). In red plastids, the complementary gene set contains specific genes (- - -) involved in protein regulatory pathways (transcriptional and post-transcriptional regulation). After Grzebyk et al. 2003 (original color figure on <http://www.coccoco.ethz.ch>).

synthetic gene that is involved in chlorophyll synthesis (*chlI*) is retained in all photosynthetic algae; this gene was transferred to the nucleus in higher plants.

Gene retention and losses in chloroplast genomes reflect evolutionary patterns

The gene loss from plastids (a process which is effectively irreversible) can provide evolutionary information about the endosymbiotic associations. There are 200 or fewer protein-coding genes in primary plastids. Whereas the number of primary endosymbiotic events is still unresolved (McFadden 2001), assuming that the number of genes in the original photosynthetic prokaryote was similar to that found in the extant cyanobacterium *Synechocystis* PCC6803 (3168 protein-coding genes (Kaneko et al. 1996)), >93% of the ancestral endosymbiont genome were lost or transferred to the host nucleus in the primary plastid lineages (Grzebyk et al. 2003). From a hypothetical ancestral plastid genome containing the whole gene set of extant plastids, the three primary plastid genomes result from specific gene losses and from parallel gene losses in two plastid lineages. Green plastids exhibit the most numerous gene losses, either specific losses or losses common to the glaucocystophyte plastid. Subsequently, patterns of gene losses occurred differently at genus level radiations within primary lineages and at radiations accompanying secondary symbiotic events. Between 1% and 10% of the remaining plastid genes were lost at genus level radiations within rhodophytes and chlorophytes. A large number of the remaining primary plastid genes were lost at radiations linked to the secondary symbiotic events. For example, 15% to 20% of the plastid genes were lost with the divergence of cryptophytes and bacillariophytes from the rhodophytes, and about 30% of genes were lost at the divergence of euglenoid plastids from chlorophytes. This analysis indicates that endosymbiotic events resulted in relatively rapid, massive gene losses in plastids, whereas the radiations within phyla were accompanied by slower and more gradual genomic erosion.

One can hypothesize that the retention of complementary core set genes in the primary plastid genomes of rhodophytes was critically important for the endosymbiotic radiation of the red lineage. In this scenario, gene retention improved the evolutionary potential of red plastids to be transferred to a collection of new, phylogenetically diverse, heterotrophic host cells. In contrast, the transfer of some of these complementary core set genes to the host nuclear genome decreased the portability of primary green plastids. Once these genes were lost in a primary plastid, a potential secondary host would have to have either retrieved them from the primary host nucleus, or obtained analogous non-plastid genes from elsewhere by lateral gene transfer.

The number of secondary endosymbioses involving green symbionts is similar to the number of "red" endosymbioses – at least three groups of protists imported plastids from green algal symbionts, whereas "red" symbioses occurred two to four times, depending on one's phylogenetic framework (Cavalier-Smith and Beaton 1999; Delwiche 1999). One might propose that green symbioses occurred

early in eukaryotic history, before plastid gene loss was completed. For the euglenophytes and chlorarachniophytes, the plastid phylogeny inferred from 16S rDNA suggests their plastids are derived early within the green plastid lineage (Bhattacharya and Medlin 1998), a conclusion supported by both the retention of the *rpl22* gene in the euglenophyte plastid genome and by fossils of photosynthetic euglenids (identified by features of lorica ultrastructure) in Ordovician rocks (Leander et al. 2001). Subsequent gene transfers from secondary green plastids paralleled those from primary green plastids (e.g., the transfer of the *rbcS* gene to the nucleus of euglenophytes). Key tests of the plastid portability hypothesis will come from genomic analyses of “green” dinoflagellates, which would not appear to be early branches on the dinoflagellate tree.

The portable plastid hypothesis also predicts that secondary endosymbioses of the “red” lineage occurred relatively late in biological history, after the importation of green plastids became genetically difficult. Fossils, however, indicate that photosynthetic heterokonts existed during the Proterozoic Eon – fossils of *Palaeovaucheria* in >1000 Ma old shales of Siberia share numerous features with extant species of the xanthophyte genus *Vaucheria* (German 1990; Woods et al. 1998), and recently discovered populations in ca. 800 million year old rocks from Spitsbergen preserve nearly the complete vaucherian life cycle (Butterfield 2000). While plastid portability may have influenced the establishment of novel photosynthetic lineages within the eukaryotes, functional biology must provide explanations for the rise of the red lineage of phytoplankton to taxonomic and ecological prominence.

Competition among the red taxa

We now consider the possible causes for the radiation of both dinoflagellates and coccolithophores, but not diatoms in the Mesozoic, and the reversal in the fortunes of the former two taxa with respect to the latter in the Cenozoic. Our models are based on the macroecological features of the three taxa derived largely from physiological studies of extant species.

One striking feature that emerges is the nutritional characteristics and differences among the three clades. Many photosynthetic dinoflagellates can assimilate dissolved and particulate organic matter, thereby supplementing their photoautotrophic metabolism. While mixotrophy is hardly unique to dinoflagellates, the extent to which they exploit this nutritional lifestyle is remarkable. Mixotrophy is advantageous under two conditions: (a) in oligotrophic environments, where (by definition) inorganic nutrients are scarce and both dissolved organic nitrogen and phosphorus are relatively plentiful, and (b) in coastal regions, which are enriched in terrestrial and/or estuarine derived particulate and dissolved organic matter. We suggest that overall rise in sea level facilitated the expansion of these two conditions through the Jurassic and Cretaceous. At the same time, the vast extent of the Panthalassic Ocean, coupled with a general sluggish thermohaline circulation,

would have contributed to an unusually widespread distribution of subtropical and tropical oligotrophic marine ecosystems.

Contemporary coccolithophores are generally mesotrophic/oligotrophic taxa, and are widely distributed throughout the subtropical seas. Two contemporary species, *Emiliania huxleyi* and *Coccolithus pelagicus*, form blooms, usually at mid to high latitudes. The blooms, which are detectable from satellite imagery (see Balch et al. this volume), are keyed to a shoaling of the mixed layer, an increase in incident solar radiation, and decreasing concentrations of nitrate and phosphate. Whereas coccolithophores are obligate photoautotrophs, available physiological data indicate that they have a relatively high affinity (low half-saturation constant) for both macronutrients. In the North Atlantic, coccolithophore blooms always succeed blooms of diatoms.

Arguably, diatoms are the most ecologically successful eukaryotic algae in the contemporary ocean. They currently are responsible for ca. 40% of the net primary production and upwards of ca. 50% of the organic carbon exported to

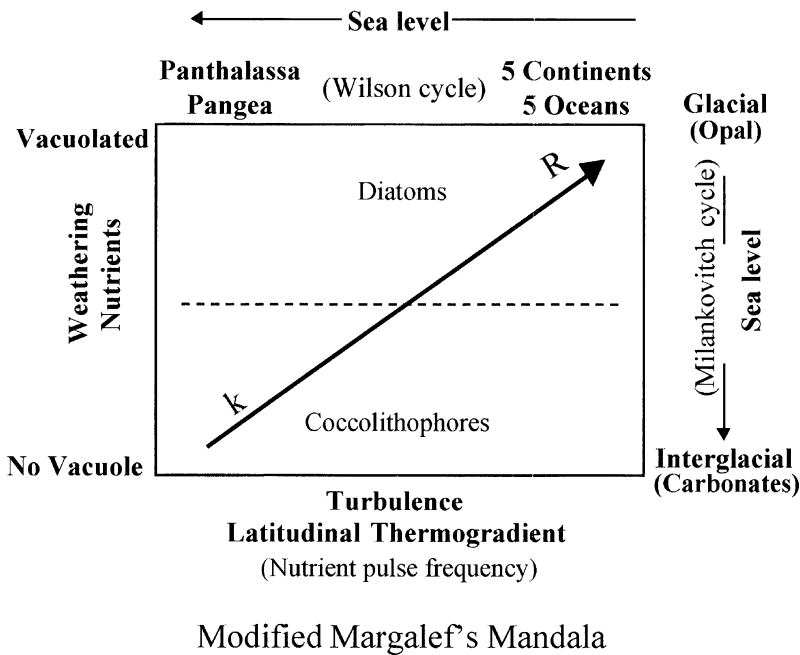


Fig. 7. An extension of Margalef's Mandala (Margalef 1997), showing that increased turbulence and subsequent increased available nutrients lead to a shift from K to r species. Turbulence and nutrient concentration in the ocean reflect the latitudinal thermogradient that is function of radiative forcing, continent dislocation and sea level.

the ocean interior (Smetacek 1999). Diatoms began their rise to prominence in the late Eocene when dinoflagellate and calcareous nannoplankton diversities began to decrease and diatom diversities began to increase at the level of genera (Fig. 7). These diversity changes correspond to a time when sea level began a long-term fall through the Cenozoic, decreasing the extent of flooded continental shelf area. This indicates that tectonics and the Wilson cycle may have played a role in diatom evolution in the Cenozoic. However, diatom evolution at the species level occurred in two pulses: 1) a dramatic radiation at the Eocene/Oligocene boundary; and 2) sustained increase in diversity through the middle and late Miocene to present time. These results suggest that tectonics and extent of continental shelf flooded were not the primary driver of diatom evolution; rather, diversity changes in diatoms appear to be linked to major changes in both Cenozoic climate and the terrestrial biosphere. What are the possible links?

Diatoms are unique among extant photoautotrophic taxa in that they have an absolute requirement for orthosilicic acid, which they polymerize on a protein matrix to form strong shells called frustules. Silica is introduced into the oceans primarily by continental weathering, but the present day surface ocean is strongly undersaturated with respect to silica as a direct consequence of diatom growth. Diatoms are basically neritic, and blooms are largely confined to continental margins, and shallow seas, and such open ocean regions as the North Atlantic and Southern Ocean where silica can be supplied through upwelling. Hence, one possible clue to the rise of diatoms in the Cenozoic may lie in an increased flux of silicic acid from the continents.

A second feature that distinguishes diatoms from dinoflagellates and coccolithophores is the evolution of a true nutrient storage vacuole. The vacuole occupies approximately 35% of the volume of the cell, and can retain high concentrations of nitrate and phosphate. Importantly, ammonium cannot be (or is not) stored in a vacuole. The vacuole allows diatoms to access and hoard inorganic nutrients, thereby potentially depriving competing groups of these essential resources. Consequently, diatoms thrive best under eutrophic conditions and in turbulent regions where nutrients are supplied in frequent high pulses. The competition between diatoms and coccolithophores can be modeled easily using a resource acquisition model based on Droop's formulation for nutrient uptake (Droop 1974). In such a model, diatoms dominate under highly turbulent conditions, when their nutrient storage capacity is maximally advantageous, while coccolithophores dominate under relatively quiescent conditions (Tozzi et al. in press). These results fit the classical *r* and *K* paradigm, where *r* strategists (e.g., diatoms) dominate in high mixing environments and *K* strategists (e.g., coccolithophores) dominate oligotrophic conditions. Although it is theoretically possible that competitive exclusion could occur under extreme conditions, it does not do so in the real ocean. The coexistence of two taxa competing for a single resource is a consequence of the dynamically unstable nature of aquatic ecosystems (Li 2002).

Recognizing these fundamental differences in physiology, Ramon Margalef proposed that competition among these three major taxa could be related to turbulence in the upper ocean (Fig. 7). The so-called Margalef "mandala," can be extended over geological time to infer selection processes that have led to the early

rise of dinoflagellates and coccolithophores followed by the rise of diatoms in the Cenozoic. Can we extend Margalef's "mandala" to a geological (and hence, evolutionary) context?

On relatively recent time-scales, such as Pleistocene, the sedimentary record reveals a periodicity of opal/calcite deposition corresponding to glacial/interglacial periods. We propose that such alterations in mineral deposition are related to upper ocean turbulence; i.e., the sedimentary record is a "fax" machine of mixing (Falkowski 2002). Glacial periods appear to be characterized by higher wind speeds and a stronger thermal contrast between the equator and high latitudes. In accordance with our simple nutrient uptake model, these two factors would favor diatoms over coccolithophores. During interglacials, more intense stratification, weaker winds, and a weaker thermal contrast between the equator and poles would tend to reduce upper ocean mixing and favor coccolithophores. While other factors (e.g., silica availability) undoubtedly would also influence the relative success of diatoms and coccolithophores on these time-scales, we suggest that the climatically forced cycle, played out on time scales of 20–100 ky (over the past 3 my), can be understood as a long-term competition that never reaches an exclusion equilibrium condition.

Can the turbulence argument be extended to even longer time-scales to account for the switch in the dominance from coccolithophores to diatoms in the Cenozoic? Even though the fossil record of diatoms in the Mesozoic is obscured by problems of preservation, several diatom species have been reported from the late Jurassic (Harwood and Nikolaev 1995), and the group could have existed much earlier (Moldowan and Jacobson 2000). However, the diatoms found in Cretaceous marine deposits almost uniformly belong to extinct stem taxa, indicating that both modern taxa and the modern importance of diatoms in export production were not established before the late Mesozoic. We suggest that the ongoing ecological and evolutionary displacement of coccolithophores by diatoms in the Cenozoic is, to first order, driven by tectonics. The Mesozoic interval was relatively warm and was characterized by a two cell Hadley circulation, with obliquity greater than 37.6° , resulting in a relatively well-mixed atmosphere. The atmospheric meridional heat transport decreased the latitudinal thermogradients; global winds and ocean circulation were both sluggish (Huber et al. 1995). This relatively quiescent period of Earth's history was ideal for coccolithophorids. However, climate conditions began to deteriorate in the mid-Eocene. Tectonic gateways opened through the Drake Passage and between Australia and Antarctica by the Eocene/Oligocene boundary (33.7 Ma) (Kennett 1977; Barrett 1996). The resulting surface and intermediate water circulation around Antarctica most likely contributed to the thermal isolation of the continent at this time. In addition, modeling studies show that declining atmospheric CO_2 levels played a major role in ice volume increase at the same time (DeConto and Pollard 2003). With the establishment of a large, permanent Antarctic ice sheet by the Eocene/Oligocene boundary, Hadley circulation changed dramatically. The net result was more intense thermohaline circulation, greater wind mixing and decreased stability (Chandler et al. 1992; Barron et al. 1995). Hence, decreased stability of the upper ocean may have contributed to the rise of the diatoms in the Cenozoic.

The isotopic record from carbonates suggests that orogenic uplift in the Cenozoic, coupled with a decline in volcanism, have further led to a long-term depletion of CO₂ and a corresponding decrease in temperature of the ocean's interior (Crowley and North 1991; Kump and Arthur 1999). These geochemically driven climate changes may have increased oceanic stratification, which has, in turn, led to an increased importance for wind-driven upwelling and mesoscale eddy turbulence in providing nutrients to the upper ocean. The ecological dominance of diatoms under sporadic mixing conditions indicates that their long-term success in the Cenozoic reflects an increase in event scale turbulent energy dissipation in the upper ocean. But was tectonics the only driver?

The coevolution of mammals, grasses, and diatoms

Through the silicate weathering cycle, orogeny may have been a catalyst in the long-term radiation of diatoms in the Cenozoic. Huh and Edmond (1999) have argued that on million year timescales, continental elevation determines weathering fluxes to the oceans. Insofar as both uplift and regression have characterized nearly all continents throughout the Cenozoic, increasing nutrients in general, and increasing fluxes of silica in particular, might have facilitated diatom expansion. ⁸⁷Sr/⁸⁶Sr values began a long-term increase (suggestive of an increase in continental weathering) near the Eocene/Oligocene boundary, at about the same time as the first major radiation in diatoms (Fig. 3). However, orogeny alone cannot explain the relatively sharp increase in diatoms at the Eocene/Oligocene boundary and in the middle through late Miocene. We must look for other contributing processes.

True grasses arose shortly after, or perhaps coincident with, the Paleocene/Eocene Thermal Maximum (PETM) (Kellogg 2000). The first definite occurrence of C₃ grass pollen has been dated as ~55 Ma (Jacobs et al. 1999). Grasses remained sparse until the Eocene/Oligocene boundary (33.7 Ma) (Retallack 2001), corresponding to a global climatic drying associated with the establishment of major glaciation in the Antarctic. The rapid co-evolution of grazing ungulates accompanied the expansion of grasses at this time, displacing browsers (Janis et al. 2002). Grasses contain up to 15% dry weight of silica, which forms micromineral deposits in the cell walls, called phytoliths. Indeed, the selection of hypsodont (high crown) dentition in ungulates from the brachydont (leaf eating) early-appearing browsing mammals coincides with the widespread distribution of phytoliths and grit in grassland forage. It is tempting to suggest that the rise of grazing ungulates and the radiation of grasses in effect was a biologically catalyzed silicate weathering process. The deep root structure of Eocene grasses certainly facilitated silicate mobilization into rivers and ground waters. The increase in phytolith diversity and abundance since the late Eocene (Retallack 2001) indicates that the mobilization of silica from soils by grasses increased as the grasses evolved and expanded. The subsequent transfer of this silica to the oceans (via fluvial and aeolian transport) must have increased the bioavailability of silica to diatoms, which also began to diversify at the genus level in the late Eocene (Fig.

3). We propose that this mechanism can account for the close correlation between evolutionary pulses of grasses and diatoms.

Short grasslands became widespread by ~17 Ma (Retallack 1997; Retallack 2001), and initially were dominated by C_3 grasses (Retallack 1997; Retallack 2001) even though C_4 grasses evolved ~15 Ma or earlier (Latorre et al. 1997). The second pulse of diatom diversification at the species level in the Neogene coincides with the expansion of grasslands coupled with a shift from C_3 to C_4 grasses in the late Miocene.

The feedback between the co-evolution of mammals and grasses and the supply of silicates to the ocean potentially explains the punctuated radiation of diatoms, and the continued dominance of diatoms in the Cenozoic. There is another potential feedback at play however, that “locked in” diatom preeminence. Indeed, one could argue that the increase in diatom dominance, and the associated increase in the efficiency of carbon burial, played a key role in decreasing atmospheric CO_2 over the past 55 My. If so, diatoms have inadvertently helped to make the ocean more turbulent, thereby potentially decreasing competition from dinoflagellates and coccolithophores. Perhaps ironically, humans, through the combustion of fossil fuels, are fleetingly interrupting this long-term process. That biological selection may influence climate is clearly controversial, however, the evident trends in succession between taxa on long time-scales, and cycles in dominance on shorter geological time-scales beg for explanation.

Conclusions

Evolution, like any other complex system with emergent properties, cannot be explained by a set of ordinary differential equations. Rather, the links among processes, which are non-linear and fluctuating, form patterns are recorded in the geological record and presumably reflect preserved patterns in ecological structure. Just as a family’s history cannot be completely understood from a photo album, we cannot look at snapshots of the geologic record and totally understand why the ocean is dominated by red eukaryotic phytoplankton while the land has been conquered by green plastids. In our analysis however, we have suggested some processes that address the three basic issues we raised.

We proposed that a major factor that contributed to the decline of the green lineage in ecological importance following the end-Permian extinction was a long-term change in the chemistry of the ocean, specifically a long-term decline in the formation of anoxic/hypoxic waters that began in the mid-Triassic. Our results from trace metal preferences reveal that, while redox states cannot fully account for the selection of the red lineage, trace element distributions keyed to suboxic conditions were probably conducive to the relative fitness of red plastid eukaryotes in the ocean. To our knowledge, this type of selection pressure did not occur in terrestrial ecosystems throughout the Phanerozoic. Once selected, red plastids appear to be much more portable than green plastids by virtue of a complementary set of genes retained in the former organelle.

Finally, we examined some of the potential process(es) that contributed to the radiation of the dinoflagellates and coccolithophores, but not the diatoms, in the Mesozoic, and why the two former groups have receded, while the last has risen to prominence in the Cenozoic. Our analysis suggests that two overarching processes played key roles in the selection of specific red taxa on these time-scales. The first is oceanic turbulence, dictated on all time-scales by the equator to pole and continent to ocean thermal gradients. This hypothesis is testable in the modern ocean on ecological times scales. We suggested that, compared with the late Cenozoic, the Mesozoic oceans were relatively quiescent, whereas over the past 34 Ma there has been an increase in upper ocean turbulent mixing. We couched these notions within the context of Margalef's "mandala" on geological time-scales. However, we further suggest that the long-term rise in fortunes of diatoms over the past ~34 myr has been, in part, due to the co-evolution of grasses, which accelerated the silicate weathering cycle, and led to an increased carbon burial, especially on continental margins. This hypothesis led to the corollary that the long-term drawdown of CO₂ and the corresponding decrease in Earth's temperature since the Oligocene has, in part, been a consequence of the rise of diatoms. This hypothesis is supported by the carbon isotope data from carbonates over the last 210 myr (Katz et al. in review). We hope that this brief essay will stimulate further exploration of the macroevolution of marine phytoplankton in the context of Earth's geological history.

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Plankton community behavior on ecological and evolutionary time-scales: when models confront evidence.

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Summary

Processes of current and past global change have been successfully identified and modeled by treating the earth as a physical or chemical system. Quantitative characterization of global change in the biota lags far behind. Units of measurement include biomass, productivity, abundance, diversity and species longevity. The response time to forcings of the physical and chemical systems range from seasons to a few thousand years. Response times of quantifiable aspects of the biosphere, however, may range from the ecological time-scale of days up to the evolutionary time-scale of millions of years.

The models used by ecologists and evolutionists focus both on characterizing the type and extent of abiotic and biotic processes acting on living and on evolving populations of organisms. The spacial and temporal scales to be considered in studies of ecological and evolutionary controls, however, are vastly different.

Investigations of the seasonal dynamics of coccolithophores document their strong correlation with changes of the physical-chemical environment (bottom-up control). A few detailed stratigraphic studies indicate that physical forcing also operated on evolutionary time-scales. Although commonly observed in living communities and laboratory experiments, the quantification of the influence of biotic forcing (top-down) by organism-organism interactions (grazing, predation, competition, infection) in ecology and particularly in paleontology, remains elusive. Difficulties in reconciling plankton diversity and longevity with commonly accepted ecological and evolutionary theory underscores the need for better understanding basic behaviors of the biosphere.

Introduction

Questions of global change on long and short time-scales have enjoyed high priority in international research agendas since at least the initiation of the International Geosphere-Biosphere Program (IGBP) in 1986. These efforts were subsequently extended and politically enhanced at the Earth Summit in Rio de Janeiro (1992) by the proposed UN conventions on climate change and biodiversity. Both of these issues are influenced by processes acting on very short (decades to centuries) and on very long (millions of years) time-scales. In this chapter, the marine plankton record is used as an example of the progress made and the problems encountered in trying to bridge this gap. We discuss mainly evidence from coccolithophores, but include the other three of the four major skeletonized marine plankton groups (diatoms, foraminifera, radiolaria). Fossils of all groups are usually abundant, globally distributed and their geological age is often known quite accurately. Two of the groups belong to the phytoplankton (coccolithophores, diatoms) and are responsible for a large portion of the oceanic primary production, and the other two (foraminifera, radiolarians) are zooplankton, i.e. consumers near the base of the oceanic food chain. All four build hard skeletons, the coccolithophores and foraminifers of calcite, the diatoms and radiolarians of opal, and together they influence the global carbon and silica cycles on short (seasonal) to long (geological) time-scales. Because these plankton groups occur in all major ocean basins, they are prime examples for the development and testing of models of the interactions between the biosphere (organismic world) and the geosphere (inanimate or abiotic world) on short to very long time-scales.

Global Systems Analyses

The recognition that the impact of human activities is becoming evident on the entire globe has spurred efforts to understand environmental processes not only on local to regional, but also on global scales. These efforts have been led by physicists, who have developed models for the global climate system (Fig. 1). These are based on the incidence, storage, transport and reflection of radiative energy, whose fluxes are given as watts per unit area and whose physical effects are measured as temperature in degrees Celsius.

Fossil air stored in ice cores shows significantly lower concentrations of this greenhouse gas during the last ice age (Delmas 1980). This discovery means that global climate change cannot be understood using only physical approaches; knowledge of the global carbon cycle and its underlying biology is also needed. Geochemists are now studying the global cycling of many climatically relevant atmospheric compounds. Models of the global carbon cycle have been continuously refined and provide quantitative estimates of the major reservoirs and their dynamics (Fig. 2).

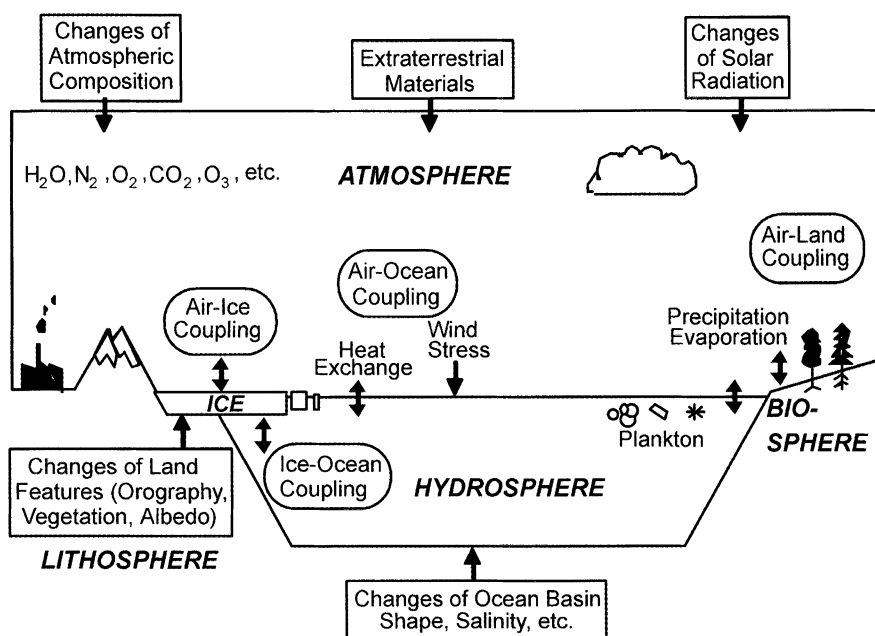


Fig. 1. Conceptual model of the global climate system. Top of atmosphere and solid earth surface are chosen as system boundaries. Processes, which influence the global climate system from outside its boundaries, are shown in square boxes, arrows and rounded boxes indicate processes and feedbacks which operate within the system boundaries.

Current geochemical models estimate that the atmosphere exchanges about one third to one fifth of its total CO₂ every year with terrestrial and oceanic reservoirs. Part of this transfer is mediated by photosynthesis and respiration. Biologists have consequently developed conceptual models of the global biosphere, its potential rates of change and its interactions with the available global physical and chemical models.

Characteristic reaction times in the physical climate system range from seasons to millennia (Ruddiman 2001). When using global geochemical models, environmental variability can reasonably be tested on the time-scales resembling those of the residence times of chemical elements in the oceans (e.g. Broecker and Peng 1982), which range from millennia to millions of years.

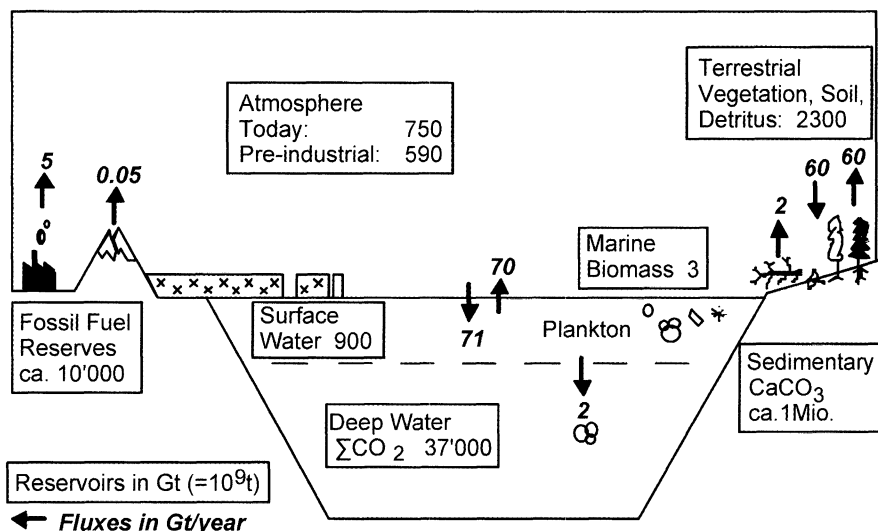


Fig. 2. Schematic depiction of the global carbon cycle (after Watson et al. 1990; Sarmiento and Gruber 2002). Shown are the major reservoirs of carbon in gigatons (square boxes) and the estimated annual exchange rates among them in Gt per year (arrows).

In contrast, ecological processes may be active on very short (e.g. days for fires or floods) to very long time-scales (e.g. millions of years as in evolution). Consequently, there has been much uncertainty and controversy in identifying parameters that may allow quantification of the global biosphere and its changes. Possible measures to characterize today's state and short-term changes of the biosphere are biomass and pigment distributions (Falkowski et al. 2000), primary production, or biodiversity. In the context of global systems analyses, characterization of primary production in units of carbon fixation per area and time would appear to be most useful (Fig. 3).

Global biodiversity, a second potentially quantitative descriptor of the global biosphere, is only moderately well known and only for certain groups of organisms. Global diversity maps have recently been compiled for terrestrial vascular plants (Fig. 4). Integrated global species richness distributions for the oceanic phytoplankton are not available. Global maps of zooplankton diversity have been compiled for planktic foraminifera (Rutherford et al. 1999) and pelagic euphausiids (McGowan and Walker 1993). Both of these groups show a general increase of diversity with increasing water temperature and both show the largest number of species in the oligotrophic subtropical gyres.

The current dearth of comprehensive measures of the global biosphere seems especially daunting, given the increasing urgency with which ecologists and organismic biologists are asked to answer to questions about interactions between species richness, habitat reduction, global carbon cycling and climate change.

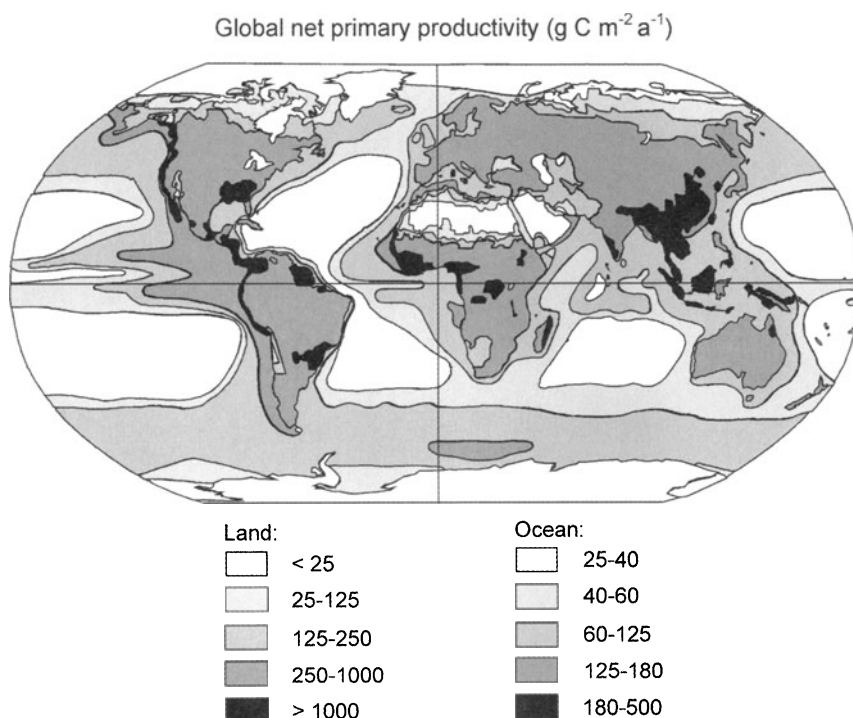


Fig. 3. Global net primary productivity distribution (after Berlekamp, Stegmann, Lieth et al. www.usf.uni-osmabrueck.de/~hlieth). Original sources: land dry mass from Lieth (1973), converted to carbon, assuming 50% of dry mass is carbon (Rankama and Sahama 1950) and ocean productivity from Koblents-Mishke et al. (1970).

Theory of Biodiversity

Biodiversity means different things to different people (Wilson 1992) and can be measured at various levels: genetic, organismic and ecologic. The two end-member descriptors of organismic diversity in space and through time are species richness (number of species) and species evenness (abundance distribution among species or equability), although various combinations of the two have commonly been used (for overview see Magurran 1988).

Because living species have all had a very long and often accidental evolutionary history, descriptors of biodiversity are needed which allow quantification on both time-scales, ecological and evolutionary. Taxon richness data is definitely the most readily available measure of biodiversity on both time-scales, although useful data are still very sparse.

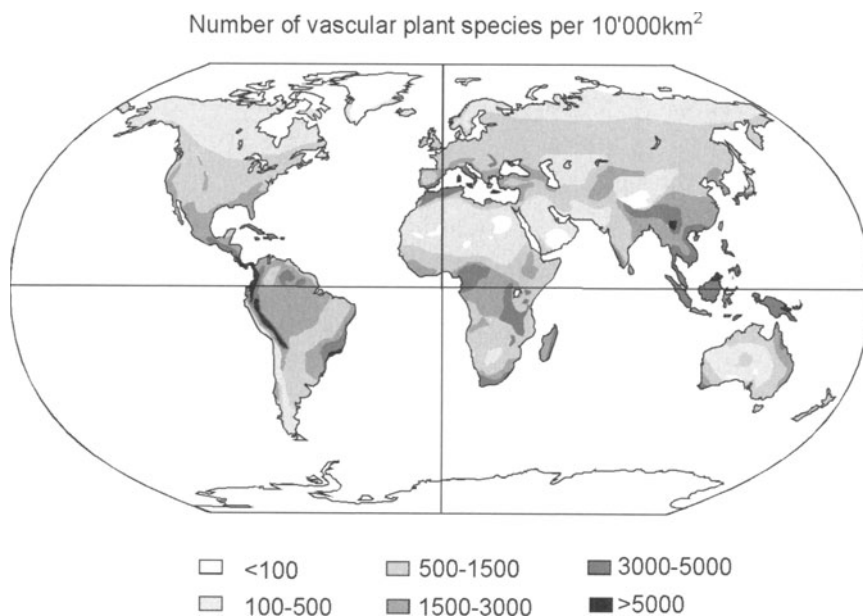


Fig. 4. Global distribution of vascular land plant species numbers (after Barthlott et al. 1999).

The two most fundamental groups of processes thought to influence or even control biodiversity are the changes in the abiotic environment (bottom-up controls) and the various interactions between organisms (biotic or top-down controls). While both of them seem undoubtedly relevant, their relative importance in any group of organisms and at any specific time in the past, is still hotly debated (Table 1). This debate has been led most fruitfully by ecologists who, through direct observations and experiments, have been able to demonstrate and occasionally quantify "bottom-up" control by relating biodiversity patterns directly to changes in biogeography, climate variability and water chemistry (e.g. Sommer 1993; Li 2002), with current emphasis placed also on changes in atmospheric chemistry, such as ozone or $p\text{CO}_2$ (e.g. Niklaus et al. 2001). The ecological effects of biotic interactions, such as competition, grazing, predation, viral infection, etc., are also being studied intensively (e.g. Verity and Smetacek 1996; Daskalov 2002). It appears that the relative proportions of abiotic and of biotic forcing may vary among different ecosystems (Worm et al. 2002).

A similar discussion on the types of processes that may have controlled evolutionary diversity changes has been carried on by paleontologists. A strong argument in favor of biotic controls was made by Van Valen (1973) in his "Red Queen hypothesis". His major argument was based on the prevalence of linearity in survivorship curves compiled for various groups through Phanerozoic time.

Table 1. Dominant processes thought to control biodiversity as proposed by ecologists and paleontologists.

Controlling processes	Ecologists	Paleontologists
Abiotic (physical/chemical variability)	Bottom-up	Stationary Model (Stenseth and Maynard Smith 1984)
Biotic (interactions among organisms)	Top-down	Red Queen Model (Van Valen 1973)

This implied nearly time constant extinction probabilities for many groups, a result that could be expected if biotic controls were dominant over abiotic ones, i.e. those exerted by changes in the physical environment. In their "stationary" evolutionary model, which was adapted from ecology, Stenseth and Maynard Smith (1984) found that a "Red Queen" outcome was theoretically possible and would express itself as largely constant speciation and extinction rates through time. If, however, speciation rate and/or extinction rate were sensitive to environmental change, a simple correlation between diversity and paleoenvironmental change should result. They concluded that only empirical evidence from the fossil record would allow discrimination between the two models. As an interesting corollary, they inferred that phenotypic stasis would be a more likely feature if their "stationary" rather than the "Red Queen" model would apply – a hypothesis that seems testable in appropriate records as well. The two models sparked a vigorous scientific debate, although conclusive evidence from the fossil record has not yet emerged.

Preservation and abundance make microfossils the most suitable group for addressing these questions of population dynamics. Paleoceanographic and related stratigraphic studies in the framework of the Ocean Drilling Program have produced numerous examples of concurrent changes in paleoenvironmental indicators, such as organic and element geochemistry, stable isotopes, sediment color, and microfossils. The impact-related mass extinction of the oceanic plankton at the end of the Cretaceous (Koeberl and MacLeod 2002) is a dramatic example. Other events of sudden changes in the physical environment, such as the late Paleocene thermal maximum (e.g. Röhl et al. 2000) or the terminal Eocene impacts of Popigai and Chesapeake Bay (Montanari and Koeberl 2000), seem to correlate with recognizable, but much less dramatic evolutionary changes of the oceanic plankton (e.g. Culver and Rawson 2000; Crouch et al. 2001).

There is a fundamental difference between the processes that potentially control biodiversity on ecological and on evolutionary scales. Ecological controls tend to act on individuals and populations of groups of species and are effective on time-scales of days to possibly decades. Evolutionary changes on the other hand are effective at species levels and thus include all populations everywhere. They are also likely to occur over time-scales of thousands to millions of years.

Processes active on ecological time-scales

Population dynamics of coccolithophores

Integrated and multidisciplinary research projects, as pursued in the framework of the International Geosphere-Biosphere Programme (IGBP), have provided unique opportunities to study the present-day interactions of living communities of organisms and their environment. In particular the ocean time series stations BATS (Bermuda Atlantic Time series Study) and HOT (Hawaiian Ocean Time series) have been the focus of multi-year, multidisciplinary sampling programs. They have provided new information on the hitherto poorly sampled oligotrophic water masses that cover one third of the global ocean's surface (Berger 1976). Although these ecosystems are characterized by recognizable seasonal changes, they are considered to be highly stable ecologically on decadal time-scales and in comparison to any other terrestrial ecosystem (Venrick 1999). As a group, coccolithophores are thought to occupy an ecological niche between diatoms and dinoflagellates with respect to mixing intensity and nutrient contents (Margalef 1978; Balch this volume).

Coccolithophores living in the top 200 m were analyzed using monthly data at BATS from January 1991 to January 1994 (Haidar and Thierstein 2001) and at HOT from January 1994 to November 1995 (Cortés et al. 2001). A major difference between these two oligotrophic areas lies in the mean abundances of the dominant taxa (Table 2). Coccolithophore cell densities (means and maxima) in the subtropical oligotrophic waters of the North Atlantic are about twice those of the subtropical North Pacific (Table 2).

Coccolithophore species richness and species evenness

The number of coccolithophore species and the frequency distribution of each species' total number of cells encountered at both sites are very unevenly distributed (Fig. 5). In the subtropical Pacific the most dominant taxa are *Umbellosphaera irregularis* (19.9%), *Emiliania huxleyi* (14.2%), *U. tenuis* (10.1%), *Florisphaera profunda* (7.3%), *Ophiaster hydroideus* (4.1%), *Discosphaera tubifera* (2.6%), *Rhabdosphaera styliifera* (2.6%), *Gephyrocapsa protohuxleyi* (2.3%), *Gephyrocapsa ericsonii* (2.2%). The remaining 35.2% of cells encountered are distributed among an additional 134 taxa each of which contributes less than 2% to the total of all cells encountered (Cortés 1998). In the subtropical North Atlantic, the frequency succession of all taxa encountered in the lightmicroscope is *E. huxleyi* (61.5%), *F. profunda* (12.9%), *U. tenuis* (4.6%), *U. irregularis* (3.7%), *Syracosphaera molischii* (3.2%), small *Gephyrocapsa* (2.8%), with all other 44 species contributing less than 2% to the total (Haidar and Thierstein 2001).

Because the cell densities at Bermuda were determined in the light microscope, reliable species assignments for many delicate or small taxa were not possible,

resulting in counts of species groups such as "holococcolithophores" or "small *Gephyrocapsa*". At the Hawaiian time series station HOT the cells were counted using scanning electron microscopy, which allowed detailed and reliable taxonomic assignments of the encountered cells. A rather detailed record of the total species richness and species evenness of all cells encountered between January 1994 and October 1996 can thus be compiled (Fig. 5). A total of 125 species and an additional 19 undescribed taxonomic units have been recognized in this two-year interval, with up to 58 species in a single sample (Cortés et al. 2001). The identified 125 species represent 63% of all 199 living coccolithophore species known, of which 137 are hetero- and 62 are holococcolithophores (Jordan and Kleijne 1994). The exceptionally high species richness and the very low species evenness, with most taxa occurring at low abundances, are very similar to the patterns observed in copepod zooplankton (McGowan and Walker 1993). It appears that plankton species may be present most of the time, albeit in such low numbers that they usually go undetected in counts of only a few hundred specimens per sample.

Table 2. Comparison of means, maxima and minima of biotic and abiotic parameters measured from 0–200 m at the time series stations BATS in the North Atlantic (Haidar and Thierstein 2001) and HOT in the North Pacific (Cortés et al. 2001) and supplementary Table S1 (at www.coccoco.ethz.ch/thierstein).

Time Series Station	BATS			HOT		
Number of Samples	217			183		
Measured Parameters	Mean	Max	Min	Mean	Max	Min
Total Coccolithophores (10^3 cells l^{-1})	20.1	105.9	<0.5	13.6	52.0	<0.5
<i>Emiliania huxleyi</i> (10^3 cells l^{-1})	13.0	92.7	<0.5	1.7	19.9	<0.5
<i>Florisphaera profunda</i> (10^3 cells l^{-1})	2.2	67.5	<0.5	2.9	15.0	<0.5
<i>Umbellosphaera irregularis</i> (10^3 cells l^{-1})	0.5	16.2	<0.5	3.1	19.9	<0.5
<i>Umbellosphaera tenuis</i> (10^3 cells l^{-1})	0.8	34.1	<0.5	1.6	16.4	<0.5
<i>Helicosphaera</i> sp. (10^3 cells l^{-1})	0.03	1.4	<0.5	0.01	0.2	<0.5
<i>Umbilicosphaera sibogae</i> (10^3 cells l^{-1})	0.1	1.6	<0.5	0.01	0.2	<0.5
<i>Syracosphaera pulchra</i> (10^3 cells l^{-1})	0.01	0.9	<0.5	0.1	0.8	<0.5
<i>Gephyrocapsa oceanica</i> (10^3 cells l^{-1})	0.03	1.4	<0.5	0.2	3.1	<0.5
small <i>Gephyrocapsa</i> (10^3 cells l^{-1})	0.7	26.2	<0.5	0.3	7.9	<0.5
<i>Calcidiscus leptoporus</i> (10^3 cells l^{-1})	0.05	2.3	<0.5	0.01	0.2	<0.5
Temperature ($^{\circ}C$)	20.7	29.6	18.4	22.9	26.7	16.8
Salinity (‰)	36.65	36.97	36.37	34.96	35.30	34.29
Nitrate (μ mole kg^{-1})	0.55	4.98	<0.1	0.53	4.16	<0.002
Phosphate (μ mole kg^{-1})	0.02	0.18	<0.05	0.10	0.34	<0.01
Light PAR ($Ein\ m^{-2}\ d^{-1}$)	5.42	42.66	<0.00	4.8	22.1	<0.02
Chlorophyll a ($\mu g\ kg^{-1}$)	0.13	0.85	<0.05	0.10	0.27	<0.02
Primary Production ($mg\ C\ m^{-3}\ d^{-1}$)	3.18	22.19	<0.5	4.01	14.62	<0.04

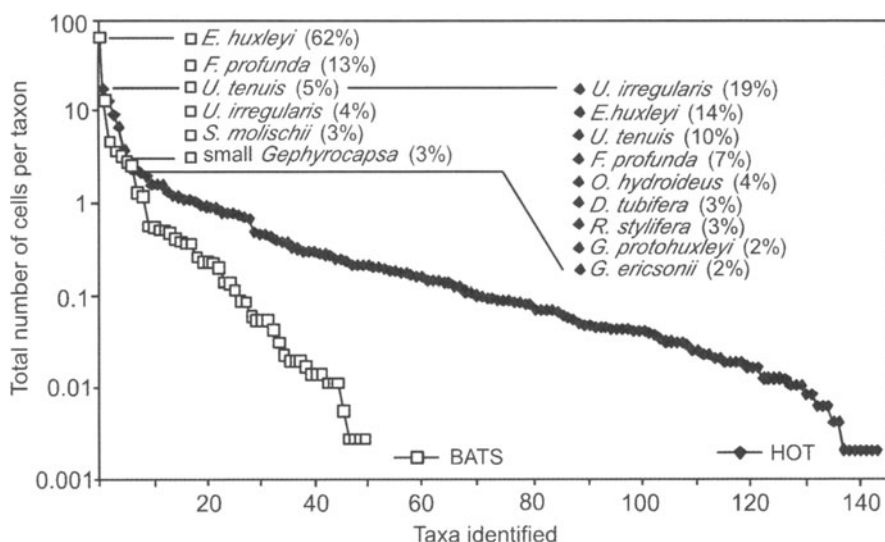
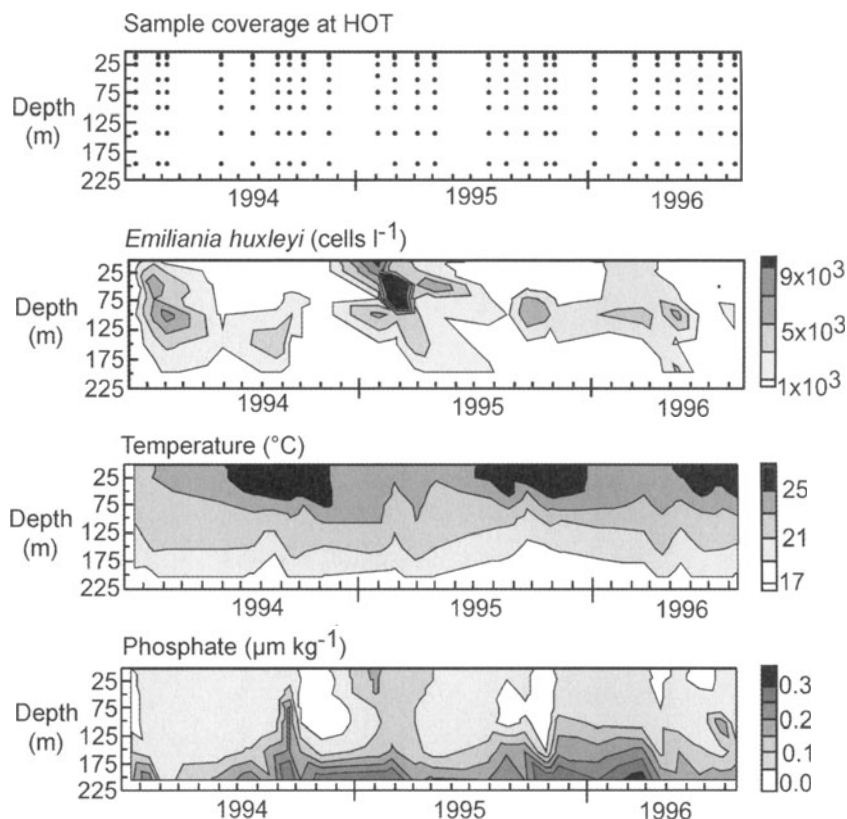


Fig. 5. Diversity of calcareous phytoplankton at Bermuda (BATS, N. Atlantic) shown as open squares and Hawaii (HOT, N. Pacific) shown as filled diamonds. Proportion of dominant taxa (relative abundance >2%) indicated for BATS on left, for HOT on right. A total of 34'996 cells were assigned to 50 taxa in the light microscope at BATS, and a total of 44'313 cells were assignable, using scanning electron microscopy, to 144 taxa at HOT (data from Cortés 1998; Haidar and Thierstein 2001; see also supplementary Table S2 at www.coccoco.ethz.ch/thierstein).

The BATS and HOT data also allowed for a test of correlations between coccolithophore cell density variabilities and environmental change, to better understand and possibly quantify the forcing of single species by certain abiotic parameters. Using multiple regression models, we can demonstrate that about two thirds of the observed cell density variability can be explained by abiotic forcing. These relationships are non-linear and we have therefore used the log of the cell densities as input into our linear multiple regression models.

Emiliania huxleyi shows distinct seasonal increases in cell densities in the upper photic zone (0–100 m water depth). Subsequently the living populations tend to gradually sink over a period of several months into the lower photic zone, where their numbers eventually decrease below detection limits (Fig. 6, upper panel). In the upper photic zone 50% of the cell density variability of *E. huxleyi* can be explained by water temperature (negative correlation) and an additional 17% of the residual variance by bioavailable phosphate (positive correlation), which sums to 67% of the variability forced by these two abiotic parameters (Fig. 6). The negative correlation with temperature is not unexpected. The highest regularly occurring seasonal cell densities observed for *E. huxleyi* tend to be in waters of about 12–14°C in the N. Pacific (Okada and Honjo 1973), N. Atlantic



$$E. huxleyi (\log \text{ cells l}^{-1})_{0-100 \text{ m}} = -0.3 \text{ Temp.} + 7.5 \text{ Phosph.} + 9.28$$

67% of total variance explained: 50% 17%

Fig. 6. As much as 67% of the cell density variability of *E. huxleyi* 1994–1996 in the 126 assemblages from the upper photic zone at Hawaiian Ocean Time series station can be explained with a multiple regression equation (bottom of figure) by the variability of temperature and phosphate (data from Cortés et al. 2001).

(Holligan et al. 1993; Broerse et al. 2000; Balch, this volume) and the Mediterranean (Knappertsbusch 1993). Since the temperature variability in the upper photic zone at the HOT station is 21–27°C, the lower end of this range is closest to the known temperature optimum of this species. A positive correlation of *E. huxleyi* cell densities with the very low phosphate concentrations (mean 0.07 $\mu\text{mol kg}^{-1}$; maximum 0.17 $\mu\text{mol kg}^{-1}$) in the upper photic zone and a negative correlation in the lower photic zone has been documented by Cortés et al. 2001).

A similarly high proportion of the cell density variability of the deep dwelling *F. profunda* can be explained by positive correlation with light intensity (53% of

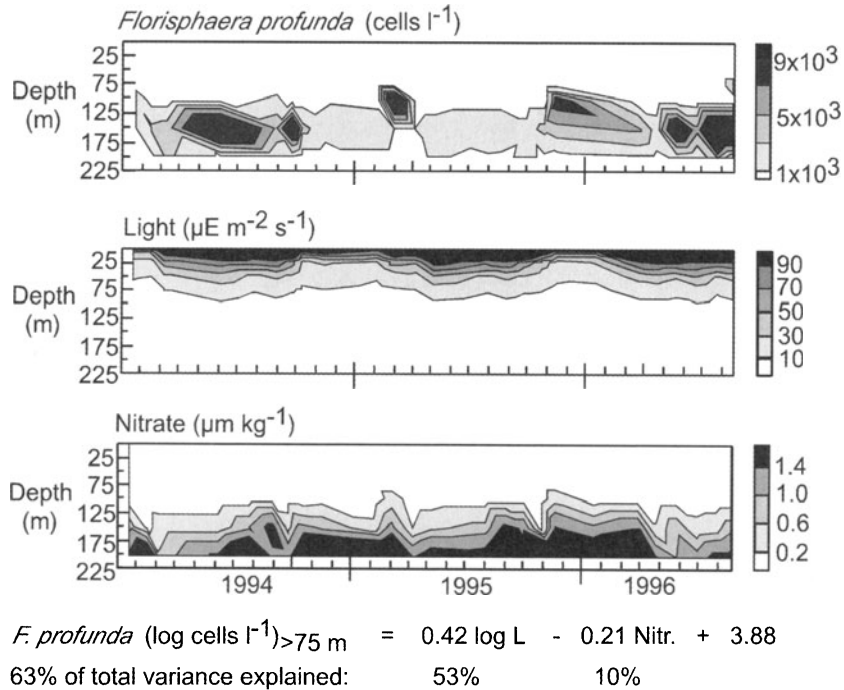


Fig. 7. Up to 63% of the cell density variability of *F. profunda* in the lower photic zone (>75 m) at Hawaiian Ocean Time series station can be explained with a multiple regression equation (bottom of figure) by the variability of light and nitrate (data from Cortés et al. 2001). For sample coverage see Fig. 6.

variance) and an additional 10% of the remaining variance by (negative) correlation with nitrate (Fig. 7). The highest *F. profunda* cell densities (i.e. >5000 cells l⁻¹) are observed at light intensities of 0.4–2.2 μE m⁻² s⁻¹ and at nitrate contents of 0.36–1.88 μmol kg⁻¹ (Cortés et al. 2001). The intersection of decreasing light with increasing nitrate appears to define the optimum ecological niche of *F. profunda* at water depths of 100–150 m at Hawaii. At Bermuda we could explain up to 46% of the observed cell density variability of single species (Table 3). The lower total correlation at BATS was likely due to the fact that the environmental measurements were not taken exactly at the same time and at the same station as the filter samples (Haidar and Thierstein 2001).

We are not aware of any other study in ecology which has been able to link such a large proportion of population variability to the abiotic forcing by temperature, light, nitrate and phosphate as we have demonstrated at the HOT station for *E. huxleyi* and *F. profunda*. The smaller proportion of abiotic forcing

Table 3. Simple (SRC) and multiple (MRC) linear regression models relating the coccolithophore cell density changes to the variability of environmental parameters (light, temperature, salinity, nitrate, phosphate) at the JGOFS time series sites BATS (Bermuda, 217 samples from the entire photic zone) and HOT (Hawaii, 120 samples in the upper photic zone (UPZ) and 43 samples in the lower photic zone (LPZ)). After Haidar and Thierstein 2001; Cortés et al 2001.

Location and species	Method	Total variance explained	Parameter and variance explained
BATS (Atlantic):			
<i>Umbellosphaera irregularis</i>	SRC	46%	Temperature 46%
<i>Emiliania huxleyi</i>	MRC	36%	Nitr. 24%, Sal. 9%, Temp. 3%
HOT (Pacific):			
<i>Emiliania huxleyi</i> (UPZ)	MRC	67%	Temp. 50%, Phosp. 17%
<i>Florisphaera profunda</i> (LPZ)	MRC	63%	Light 53%, Nitr. 10%

obtained near Bermuda in comparison to Hawaii may be caused by either a larger influence of biotic interactions, possibly grazing or infection, but also by the fact that the environmental parameters were measured at a location about 40 km away from the plankton sampling site, and were offset by two weeks from the latter (Haidar and Thierstein 2001). At the Hawaiian time series station environmental parameter measurements and plankton filtration took place on the same day and thus may be more representative than the BATS data. The remaining 33% cell density variability not accounted for by the changes in the measured set of environmental variables may be due to forcing by abiotic parameters not monitored, to biotic interactions, or to randomness.

There are apparent similarities between Pacific and Atlantic coccolithophores in the niche partitioning of the most abundant and characteristic taxa (Fig. 8), although all coccolithophores tend to co-occur within a relatively narrow environmental parameter range.

Species richness of other skeletonized plankton groups

The ecological dependency of species populations of other plankton groups have also been analyzed in regional studies of living plankton samples and in comparisons of species abundances determined in surface sediment assemblages with surface water characteristics. For the planktic foraminifera, a total of 45 living species are known (Hemleben et al. 1989), of which 34 have also been identified as skeletons in Holocene deep-sea sediments (Rutherford 1999). A quantitative relationship between species richness and surface water temperature

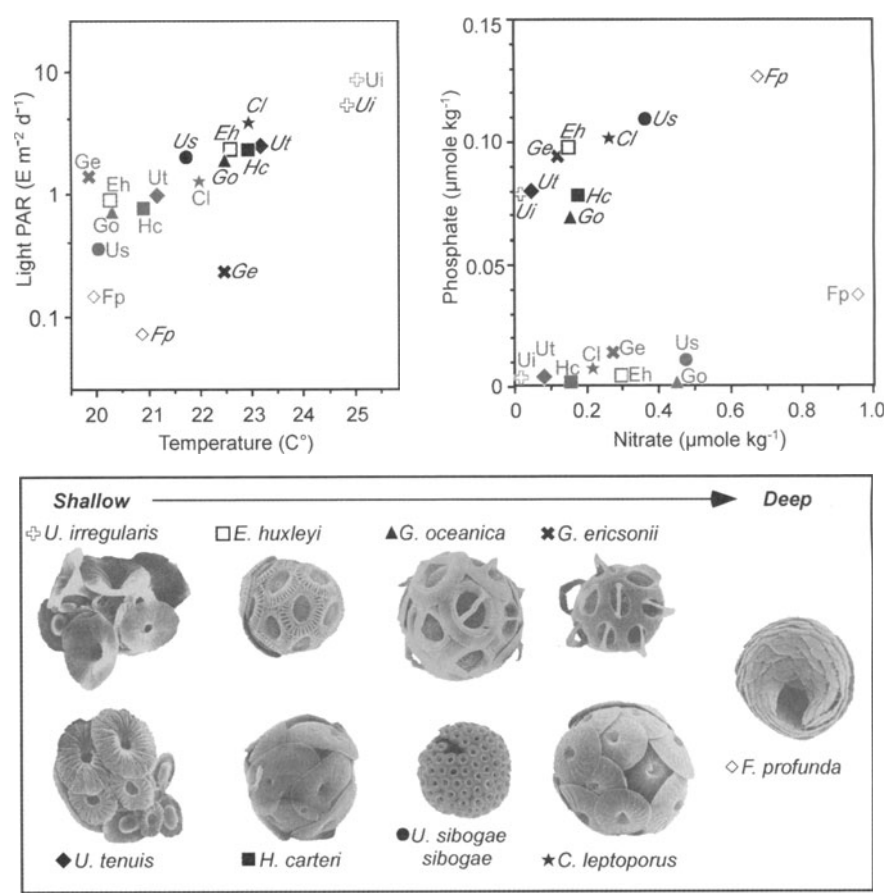


Fig. 8. Weighted mean cell densities of nine dominant and CODENET species at BATS (black italics) and HOT (gray upright) with respect to light (PAR = photosynthetically active radiation) and temperature (upper left graph), nitrate and phosphate (upper right graph), and depth succession of taxa (lower graph). Data from Haidar and Thierstein (2001) and Cortés (1998). Micrographs from www.emidas.ethz.ch.

has been established (Rutherford et al. 1999). Species richness shows a quasi-logistic increase from polar to subtropical temperatures and decreases again at higher temperatures (Fig. 9). A secondary control appears to lie in the degree of surface water stratification and its seasonal variability.

Much less is known of the global species richness of the siliceous plankton groups, since most published studies and compilations have been of regional extent. For radiolarians, the most comprehensive diversity compilation was obtained from a study of sediment trap material in the equatorial Atlantic and Pacific (Takahashi 1991). His list contains 258 formally described polycystine

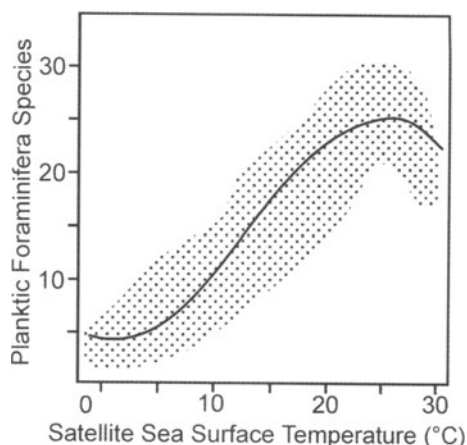


Fig. 9. Nonlinear relationship (solid line) of planktic foraminifera species richness (data in shaded area) in Holocene surface sediments of the Atlantic Ocean with satellite derived sea surface temperatures (from Rutherford et al. 1999).

radiolarian species (supplementary Table S3 at www.coccoco.ethz.ch/thierstein), which is considerably more than the 155 polycystine species listed in a more recent survey from the South Atlantic (Boltovskoy 1999). No comprehensive taxonomic list of living oceanic diatoms is currently available. We therefore compiled a list of a total of 592 species from a number of studies of the living plankton and Holocene surface sediments that have routinely been recorded in the reports of the Deep-Sea Drilling Project (DSDP) and Ocean Drilling Program (ODP) (details in supplementary Table S4 at www.coccoco.ethz.ch/thierstein). This total is slightly higher than the estimate of about 500 (including benthics) given in Schrader and Schuette (1981).

Preservation factors in species richness data

How well do the species inventories of the living plankton compare with the skeletonized records preserved in deep-sea sediments? Using species inventories published in the numerous reports of the Deep-Sea Drilling Project and Ocean Drilling Programme (Spencer-Cervato 1999) a comparison of the representativeness of the Holocene sedimentary evidence with the established biological diversity can be assembled (Table 4). The proportion of living species which are also reported from deep-sea sediment assemblages ranges from a low of 17% for diatoms to a high of 87% for the planktic foraminifera. The discrepancies are likely due to post-mortem dissolution. Diversity changes seen in the geological record must therefore be interpreted with caution.

Table 4. Number of living and fossil species reported for major skeletonized plankton groups and preservation factors (fraction of living species also reported as fossils). For data on living heterococcolithophores and planktic foraminifera see text, for living diatoms and radiolaria see supplementary tables S3, S4 at www.coccoco.ethz.ch/thierstein, and for species reported as fossils see Spencer-Cervato 1999.

	Species reported living in surface waters	Extant species documented in DSDP/ODP holes	Preservation factor
Heterococcolithophores	137	33	24%
Diatoms	592	103	17%
Foraminifera	45	39	87%
Radiolaria	258	114	44%

Processes active on evolutionary time-scales

The methods used for the analyses and reconstructions of the evolutionary history of the oceanic plankton have been the same as applied in other fossil groups. They include species richness compilations, their components (rates of speciation, extinction and turnover) and species longevity analyses (see also Bown et al. this volume). Compared to other groups of organisms, there are several distinct advantages that characterize the plankton record, such as global geographic sample coverage, rather precise age dating, and availability of numerous specimens in each assemblage, allowing for statistical analyses of composition, sizes and shapes of entire populations and of frequent species. Paleogeographic and stratigraphic analyses of population dynamics at various levels of resolution also allow the determination of species evenness (abundance ranking of species), an additional potentially useful paleoecological and evolutionary parameter.

Population dynamics

The high correlation of plankton population changes with surface water temperatures and their application to climate history was pioneered 70 years ago by Schott (1935). Increasingly detailed and sophisticated methods and calibrations since then have all relied on the assumption of direct or indirect forcing of species abundances by changing watermass properties, with temperature featuring as the most prominent parameter (e.g. Imbrie and Kipp 1971; CLIMAP 1976; Fischer and Wefer 1999).

A prominent second example for abiotic forcing of plankton change has been the mass extinction of the two calcareous plankton groups (nannofossils and planktic foraminifera) at the Cretaceous/Tertiary-boundary. The temporal coincidence of species disappearance, noble element enrichment, thin layers of tectites, shocked quartz and charcoal and the identification of a plausible impact crater, have led to wide-spread acceptance of a bolide impact hypothesis for this event (Alvarez et al. 1980; Hildebrand et al. 1991; Koeberl and MacLeod 2002). Although the coincidence of the impact tracers with the biotic turnover is well documented only for the calcareous plankton (Koeberl and MacLeod 2002), the event must have affected many other groups of organisms with comparable severity. It is possible that the more limited abundance, distribution and preservation of their fossilized record may not ever allow documentation at a comparable resolution. The evidence for other abiotic forcing mechanisms, such as large-scale volcanism (Courtillot 1994), methane release (Pálfi et al. 2001), climate and related sea-level change (Bown et al. this volume), etc. have so far remained controversial and may likely have exerted a more gradual influence on extinction and speciation rates.

Species richness

Species richness is the most simple of all diversity measures but unfortunately, it may be biased by differential preservation of microfossils (see previous section) and by monograph effects. While details of the stratigraphic species richness plots of particular plankton groups keep changing with the growing data base, the observed time intervals of high species richness (polytaxy in late Cretaceous, Eocene, Neogene) and of low species richness (oligotaxy in mid-Cretaceous, Danian, Oligocene) have been known for a long time (e.g. Tappan and Loeblich 1972; Fischer and Arthur 1977). In the Mesozoic the species richness changes of planktic foraminifera and coccolithophores seem rather gradual (e.g. Leckie et al. 2002; Bown this volume). At some of the oceanic anoxic events they mask, however, relatively high evolutionary turnover rates when speciation and extinction rates of planktic foraminifera seem to have increased in concert. A far better correlation of species richness with environmental change is observable in the Cenozoic in both calcareous plankton groups (e.g. Lipps 1970; Wei and Kennett 1983; Norris 2000; Bown et al. this volume). Stratigraphic resolution and taxonomic documentation of radiolarian and diatom diversity changes are still insufficient for any detailed comparisons with known paleoceanographic events although claims for such correlations have been made.

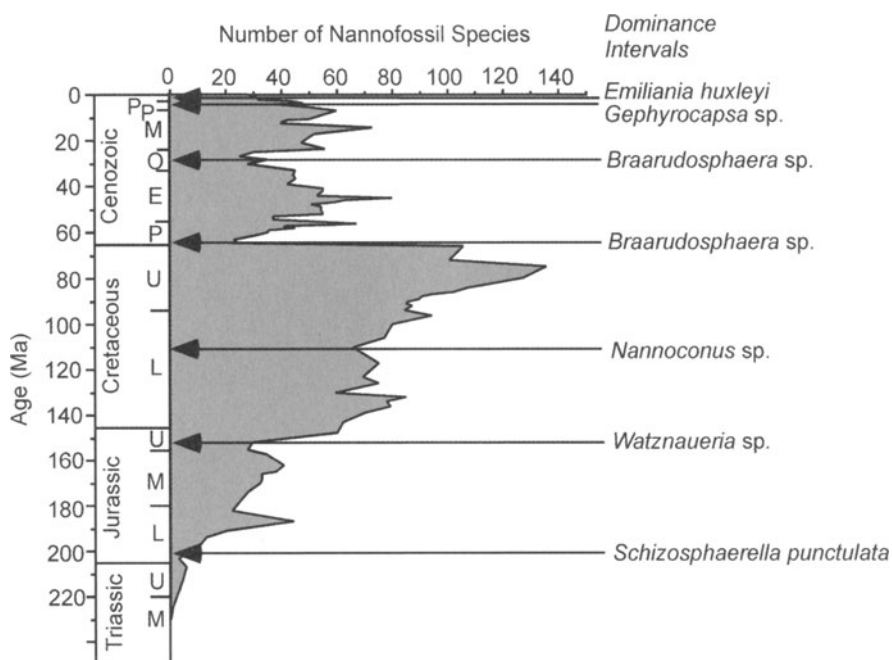


Fig. 10. Nannofossil species richness and dominance intervals discussed in text. Species numbers for each nannofossil zone or substage are taken from Bown et al. (1992) and calibrated to chronologies in Berggren et al. (1995).

Species evenness

Intervals of regional to global dominance of single coccolithophorid taxa have been known for a long time (Fig. 10). In the early Jurassic *Schizosphaerella punctulata* has been known to dominate nannofossil assemblages in many Liassic assemblages (Busson and Noël 1991). Nearly monospecific *Watznaueria* sp. assemblages have been reported world-wide in the latest Jurassic (e.g. Gallois 1976; Bralower et al. 1989) in an interval, which coincides with the rapid descent of the calcium carbonate compensation depth (CCD) in the North Atlantic (Thierstein 1979). The rise to dominance of *Watznaueria* sp. in the late Kimmeridgian and early Tithonian is also the likely cause of the transition to carbonate dominated pelagic sedimentation of the Majolica and Biancone formations in the western Tethys (Garrison and Fischer 1969). A prolonged interval of nannoconid dominance has been documented in many Tethyan sections, although its rather abrupt termination (nannoconid crisis) has so far been the major focus of attention (Erba 1994). In the Paleogene monospecific *Braarudosphaera* sp. dominance intervals have been identified at the base of the

Danian (e.g. Thierstein 1981) and in the Oligocene (e.g. Siesser et al. 1992 and references therein). In the Neogene several prominent *Gephyrocapsa* sp. dominance intervals have been described (Gartner 1977), the most recent and best documented of which lasted for about 220 ka (Bollmann et al. 1998) and ended only 260 ka ago (Fig. 10). Its underlying cause remains a mystery. The increase in the relative abundance of *Emiliania huxleyi* coccoliths observed in many deep-sea cores in the past 50 ka (Thierstein et al. 1977) may be considered the actualistic example of the beginning of another of these global dominance intervals.

It is interesting to note that these dominance intervals generally occur within oligotaxic (low diversity) intervals (Fig. 11), a fact that has earned some of them the attribution to the group of opportunistic taxa (Fischer and Arthur 1977). Such dominance intervals seem to have started after the global decrease in species richness and thus appear to be a consequence rather than a cause of oligotaxy (Fig. 11). The latter would have indicated that forcing by the biotic process of competition might have been operating.

Contrary to what one might expect from ecological models of interspecific competition, there is so far no indication that any other species went extinct during these dominance intervals. One could consider this as the evolutionary analog to the "paradox of the plankton" (Hutchinson 1961) in ecology, which appears still unresolved (e.g. Schippers et al. 2001). The paradox is seen in the conflict between the observed high species richness of aquatic systems and the results of equilibrium and competition based ecological models, which would predict much smaller diversities.

Longevity of major plankton groups

Longevity analyses have enjoyed two steps of increasing popularity among paleontologists. The first followed the Red Queen hypothesis (Van Valen 1973), which was based on the surprisingly stable longevity curves among different groups of organisms, interpreted as absence of significant influences of environmental change and prevalence of effects of biotic interactions on the evolutionary patterns of Phanerozoic organisms. A second rise in interest stemmed from the more recent concerns of declining biodiversity and its potential consequences (e.g. May et al. 1995; Heywood and Watson 1995). Considering the substantial differences in the biology, ecology and evolutionary history of the major skeletonized marine plankton groups, and the unique quality of their fossil record, differences in evolutionary strategies, extinction risk and thus longevity would be expected.

In a recent analysis of the stratigraphic occurrences of coccolithophore, diatom, planktic foraminifera, and radiolarian species reported in 176 DSDP and ODP holes with well dated chronologies, longevitys of single species and means for entire groups have been calculated (Spencer-Cervato 1999). For the 289 extant plankton species the earliest reported appearance in any of the 176 drill holes was

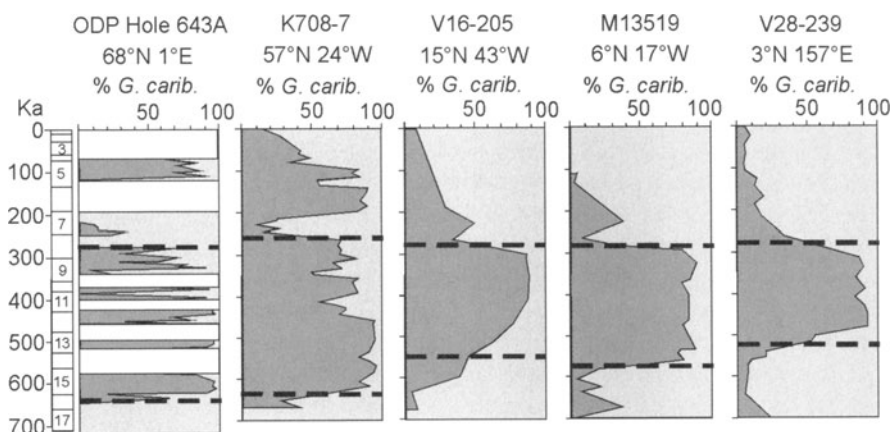


Fig. 11. Relative abundance of *Gephyrocapsa caribbeanica* (dark pattern) in Late Pleistocene deep-sea records (redrawn from Bollmann et al. 1998). *G. caribbeanica* consists of the two extant morphotypes of *Gephyrocapsa*, GO (oligotroph) and GT (transitional). The *Gephyrocapsa* dominance interval, during which >50% of all coccoliths belong to these two morphotypes, is delineated by the dashed lines.

determined. For the 1129 extinct Cenozoic species the time duration between the earliest and last occurrence was computed and the averages per group calculated (Table 5).

The results are surprising in two respects. First, the average species longevity of all taxa (extant and extinct) was about 13 million years. This is considerably longer than the commonly reported ranges of 5–10 million years for extinct species (Heywood and Watson 1995, p. 232). The optimally documented and well dated longevity of the extant species of the major plankton groups is 14.3 million years, which can be considered the half life time, since all are alive today. Second, the compiled mean longevities among the different plankton groups are similar and range only from 10.2–13.1 million years for extinct species (Table 5). Among the extant species the longevity of coccolithophores stands out with 17.8 million years, because three of the extant species have early Cenozoic and Cretaceous origins (*Coccolithus pelagicus*, *Scapholithus fossilis* with its living synonym *Calciosolenia murrayi*, and *Braarudosphaera bigelowi*). We speculate, that these extended longevities and their apparent similarities among very different plankton groups (phyto- and zooplankton, with calcitic and opaline skeletons) may be related to the spacial continuity and long-term stability of open ocean environments. The highest stability and continuity of any environment on Earth is found in the deep sea and interestingly the known species longevities of benthic foraminifera have been estimated at 16 million years for shelf faunas and at 26 million years for bathyal and abyssal species (Buzas and Culver 1984).

Table 5. Average longevitys for 1418 Cenozoic marine plankton species with well-known ranges compiled from the NEPTUNE data base (Spencer-Cervato 1999).

Plankton Group	Number of species in data base	Number of extant species only	Mean longevity of extant species (my.)	Mean longevity of extinct species (my.)
Coccolithophores	389	33	17.8	13.1
Diatoms	281	103	14.4	10.2
Foraminifera	383	39	13.9	11.1
Radiolaria	365	114	13.4	12.9
All groups	1418	289	14.3	11.9

Recent genetic analyses of living species, which have all been defined using traditional morphological criteria, show that genetic diversity may be much higher than previously thought (de Vargas et al. this volume). Evolutionary analyses to date, therefore, may have been addressing "super-species". We can expect future work to use narrower definitions of species, which will consequently result in shorter species longevitys and higher species richness.

Conclusions

Ecological knowledge has immensely contributed to a better understanding and interpretation of the evolutionary history of today's biota. However, we have illustrated in a few examples and comparisons from the comparatively well-documented plankton record, that there are potential pitfalls and limitations in simplistic extrapolations of ecological knowledge to evolutionary time-scales and interpretations. These comparisons include the quantification of the proportions of abiotic versus biotic controls on species abundances and species richness, the causes and effects of single species dominance intervals, and the surprisingly long and similar average species durations of major plankton groups. We conclude that a fresh and focused look at the biology and ecology of living organisms from a paleontological and evolutionary perspective holds great potential for adding insights related to the past history of organisms. An understanding of the biodiversity of the living biota and its natural and man-made controls, on the other hand, will never be complete without knowledge of the evolutionary history of the individual taxa. That history is preserved in the geological record and only through its analysis can we expect to gain the necessary insights into the long-term controls and the vagaries and contingencies of past environmental and biotic change.

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Calcareous nannoplankton evolution and diversity through time

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Summary

Planktic microfossils arguably provide the most complete (stratigraphic and taxonomic) record of biodiversity of any group of organisms. The phytoplankton record is of particular significance as it most likely tracks global changes in the climate-ocean system and, in turn, influenced biodiversity and productivity of higher trophic levels of the biosphere. Coccolithophores and associated calcareous nannoplankton first appear in the fossil record in Upper Triassic sediments (~225 Ma) and, despite significant extinctions at the Triassic/Jurassic boundary, the Mesozoic diversity record is one of relatively uniform increase punctuated by short periods of turnover and decline. Rates of speciation that are significantly above background were restricted to the Late Triassic, Early Jurassic and Tithonian-Berriasian intervals. Enhanced rates of extinction occurred at the Triassic/Jurassic, Jurassic/Cretaceous and Cretaceous/Tertiary boundaries.

There is no clear correlation between coccolithophore diversity and Mesozoic climate, as it is currently understood, but the dominant trajectory of diversity increase suggests long-term stability and widespread oligotrophy in photic zone environments. The Neocomian and Campanian–Early Maastrichtian intervals of diversity increase are clearly associated with increased numbers of endemic taxa at both low and high latitudes. These intervals have been interpreted as periods of cooling or cooler climates, and greater differentiation of the photic zone environment may have led to the biogeographic partitioning. Notably, none of the mid-Cretaceous Oceanic Anoxic Events are associated with above-background evolutionary rates or significant taxonomic loss or innovation.

Cenozoic nannoplankton diversity patterns are markedly more variable than those of the Mesozoic, and rates of speciation, extinction and turnover are consistently higher. There is also good correlation between diversity and climate trends, with higher diversities associated with warm climates. This is best illustrated by

the Paleogene record, where the Cenozoic diversity maximum, at the Paleocene/Eocene boundary, coincided with climates of extreme warmth, and significant diversity decline tracked climate cooling through the Late Eocene and into the Oligocene. This relationship between climate and coccolithophore diversity is contrary to that observed in the Mesozoic, and suggests different controls on evolution during the two eras. The Cretaceous record suggests that cooling within a greenhouse-mode climate system may have stimulated diversification at all viable latitudes via biogeographic partitioning. In contrast, the Cenozoic data indicates that cooling tended to drive diversity decrease. This may be explained by the greater magnitude and longevity of Cenozoic cooling, in an icehouse-mode climate system, which prevented coccolithophore diversification at higher latitudes where, instead, diatoms became established as the dominant group of phytoplankton.

Introduction

Coccolithophores and associated calcareous phytoplankton are the most abundant calcifying organisms inhabiting our planet. Their fossil record is equally impressive, perhaps being the most abundant and stratigraphically complete of any fossil group. As such, they provide a valuable proxy for Mesozoic–Cenozoic phytoplankton health and robust data concerning rates and patterns of evolutionary change. Their present biogeography and diversity is closely correlated with climatic and oceanographic zones, and their fossils provide a record of this relationship through time.

This paper will review our current understanding of calcareous nannoplankton evolution through their 225 million year history (Triassic–present) using stratophenetic phylogenetic models and a new synthesis of diversity data and rates of evolutionary change. We will discuss the adequacy of the nannoplankton fossil record, specifically potential preservational bias introduced by size, delicacy and life-cycle coccolith dimorphism. Finally, we will examine the temporal relationship between evolutionary events and the ocean-climate system and discuss the possible controlling mechanisms on nannoplankton speciation, extinction and diversity.

Methodology

Phylogenetic trees

The phylogenetic trees we present here (Fig. 1), one for coccolithophores and one for nannoliths, are based on large stratigraphic databases which have resulted from the extensive use of nannofossils in academic and industrial biostratigraphic studies over the last 50 years. Taxonomic concepts are based largely on morphological

observations of coccoliths and nannoliths using light and scanning electron microscopy: a recent review of generic and higher taxonomy was presented in Bown and Young (1997) and Young and Bown (1997). Nannofossil biostratigraphy has provided a data-set that is stratigraphically continuous and geographically widespread across the spectrum of marine environments, thus minimizing well-documented stratigraphic biases, such as preservation and sample failure, and the modified stratigraphic range phenomena that often result from these (range extension, ghost lineages, Lazarus taxa).

Although basic phylogenetic logic has been used to predict relationships between taxa, a formal cladistic analysis of coccolithophore relationships has not been undertaken. This might seem a strange omission, given both that individual coccoliths show great complexity, offering a wide range of potential characters, and that assemblages with exquisite preservation are known from many stratigraphic levels. However, there is a fundamental problem of lack of homology between different groups. This is obviously the case when comparing any nannoliths and coccoliths but also holds between coccolith groups. The V/R model (see Young et al. 1992; Young et al. this volume) provides a basic framework for interpreting homology but also highlights the degree to which different coccolith families have adopted these fundamental building blocks into intrinsically different structures. Furthermore, it is clear that coccolith structure simplification, with concomitant character loss, is a common pattern in coccolithophore evolution. One good example is the evolution of the Noelaerhabdaceae from the Prinsiaceae, which involved reduction of the V-units to vestigial nuclei, and consequent loss of characters associated with the previous, complex V-unit form. Analogously, evolution of the Calcidiscaceae from the Coccolithaceae (which is well supported by both molecular and paleontological data) involved structural simplification, with loss of the complex inner tube-cycles. Since a basic assumption of parsimony analysis is that character loss is unlikely (e.g. Smith 1994), this type of pattern further compromises cladistic analysis of the group.

Essentially, coccolith morphology is too plastic to provide sufficient robust characters for a cladistic analysis of the complete group. This does mean that, whilst we have very high confidence that the conventional families represent phylogenetic units (because the included taxa share morphological characters and/or have continuous stratigraphic ranges with plausible stratophenetic linkages), predictions of relationships between them are tenuous. Molecular genetic data (Saez et al. this volume) have broadly confirmed this pattern: conventional family- and genus-level groupings have been well-supported, but some surprising relationships between them have been revealed.

Diversity

Nannoplankton diversity has been compiled from published stratigraphic data, and notably a number of comprehensive works of synthesis (Aubry 1984; Perch-Nielsen 1985a, b; Aubry 1988, 1989, 1990, 1998; Bown 1998a; Aubry 1999). This

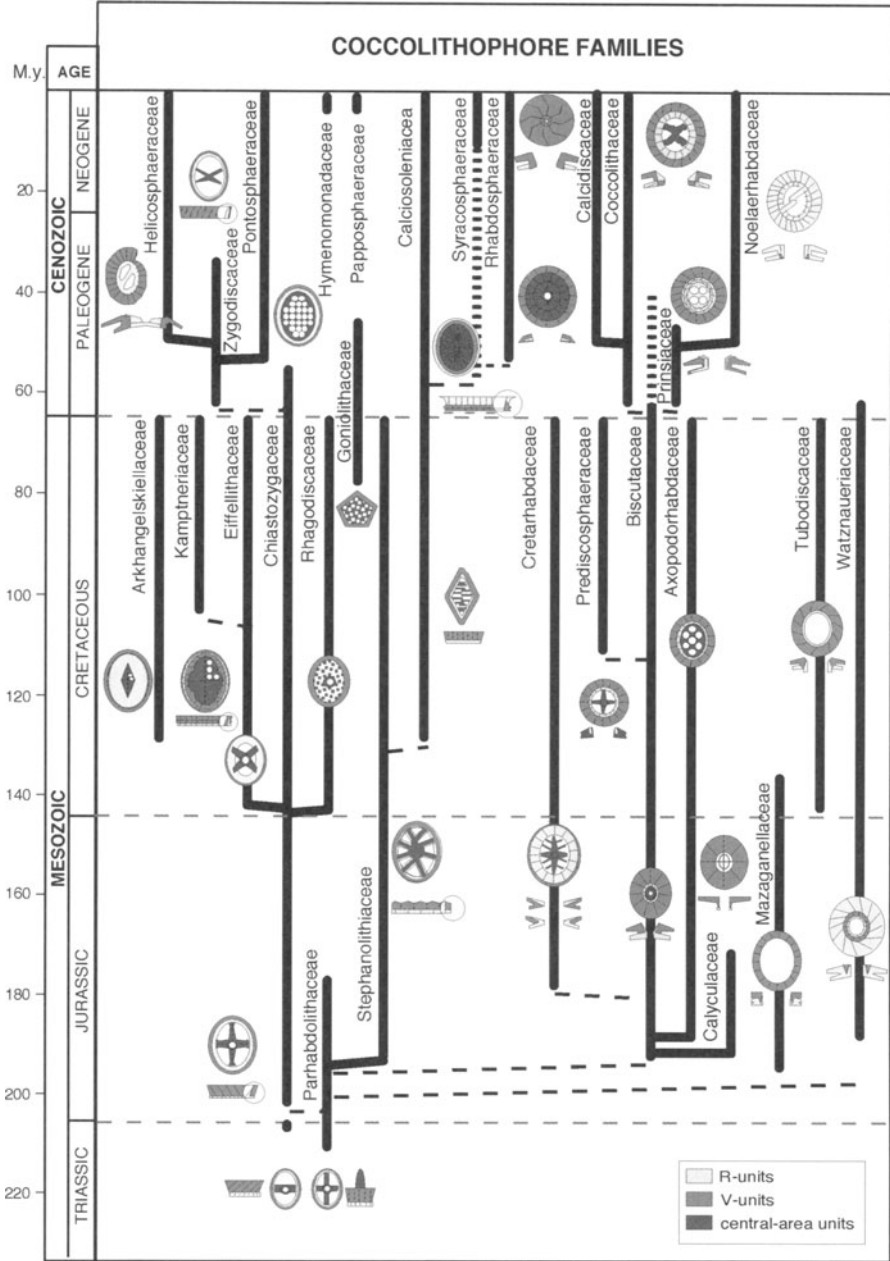
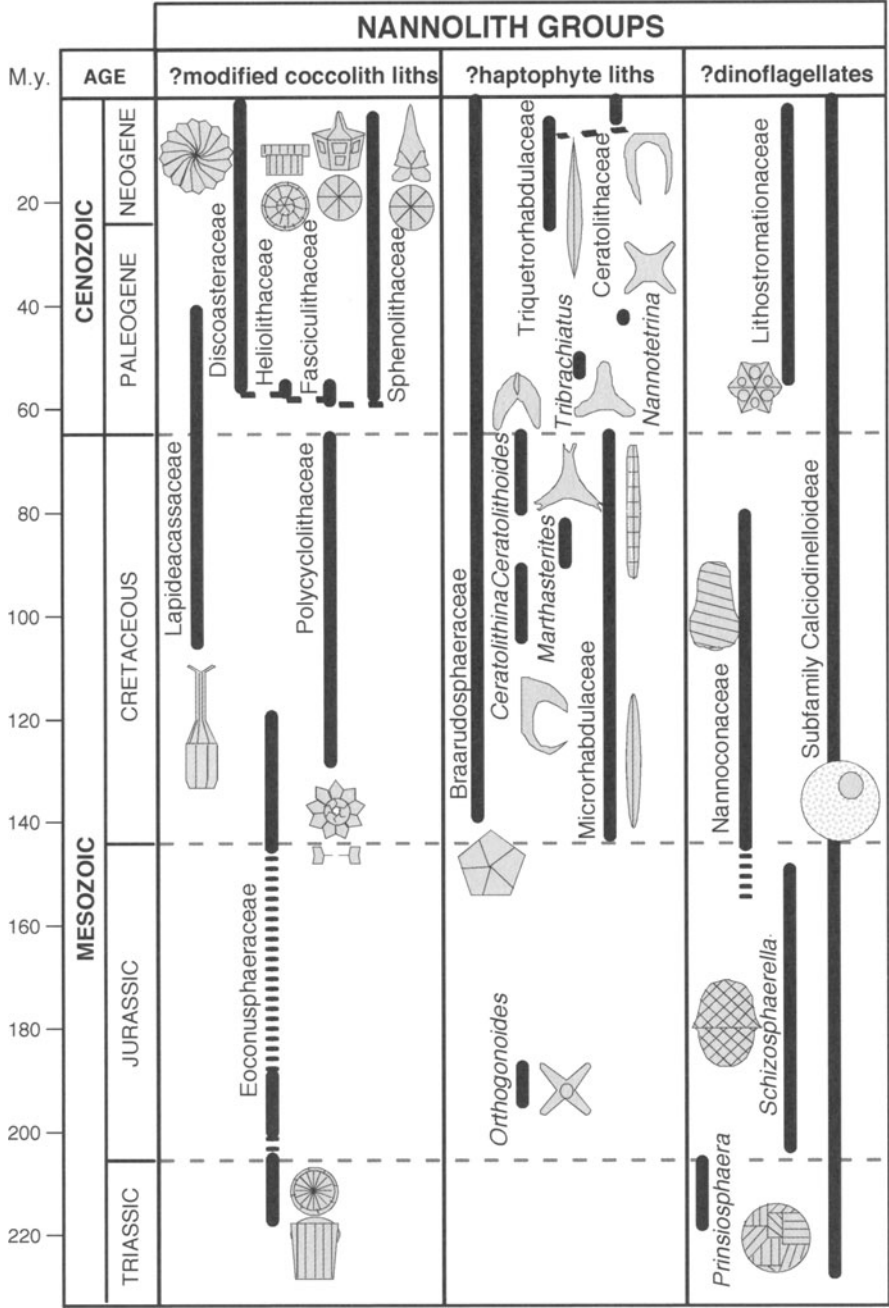


Fig. 1. Coccolithophore and nannolith family-level phylogeny.



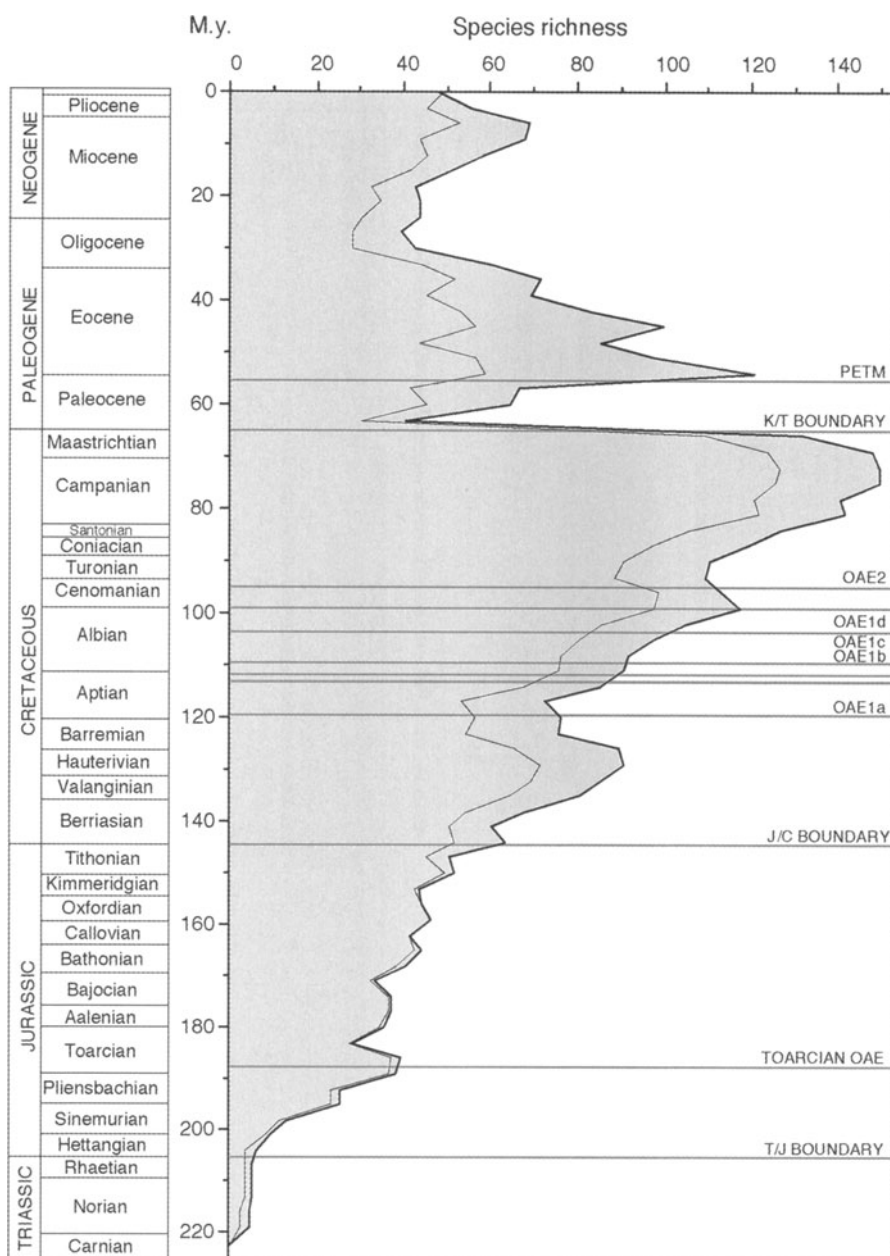


Fig. 2. Coccolithophore (light line) and total nannofossil (dark line) diversity. Data represents species richness for a three million-year interval, plotted at the mid-point. The time-scales are from Berggren et al. (1995) and Gradstein et al. (1995). OAE: oceanic anoxic event, PETM: Paleocene/Eocene thermal maximum.

published information has been screened in order to provide a consistent and informed taxonomy, and an attempt was made to reduce monographic and taxonomic-oversplitting effects. Taxa recorded from just one sample have largely been rejected, although this was often restricted to particular families, e.g. Braarudosphaeraceae and Rhabdosphaeraceae. Morphotypes which are thought to represent intraspecific variation, especially within taxa which have living representatives with polymorphic coccospheres, e.g. *Scyphosphaera*, have also been omitted. For

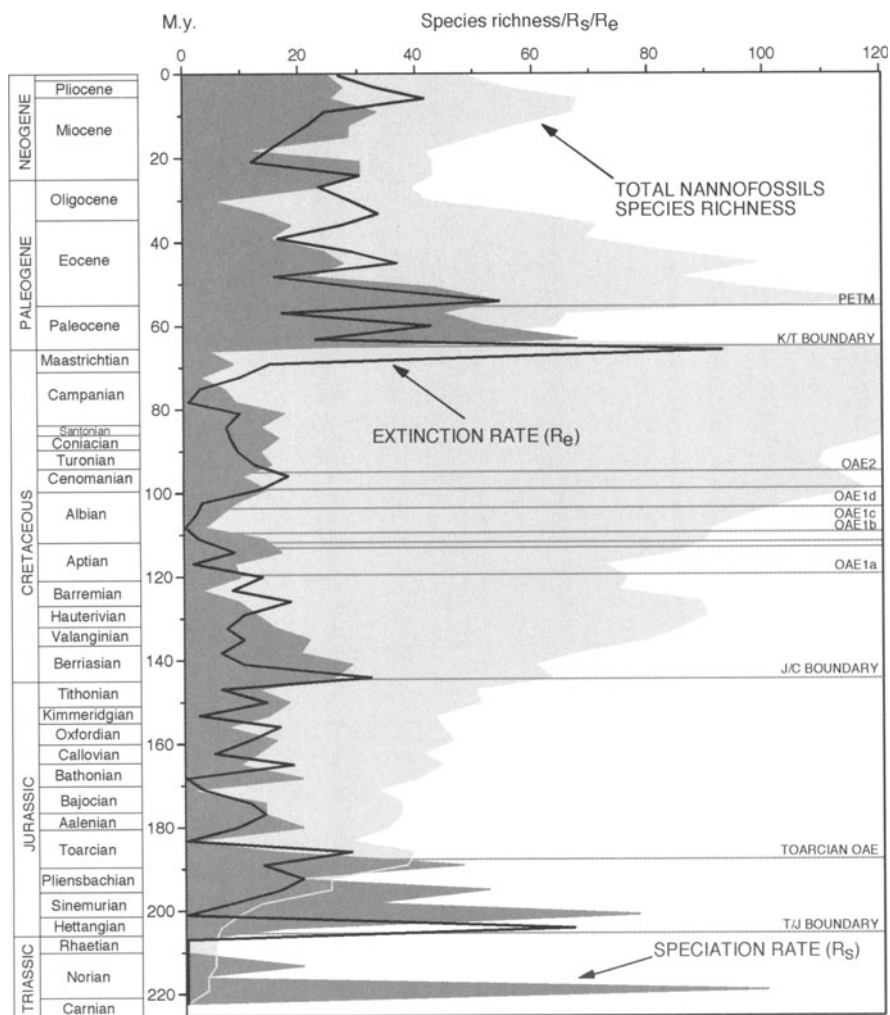


Fig. 3. Nannofossil diversity (pale shading), extinction rate (dark line) and speciation rate (dark shading). R_s/R_e represent % increase/decrease per 3 million year interval. Data plotting, time-scale and abbreviations as in Fig. 2.

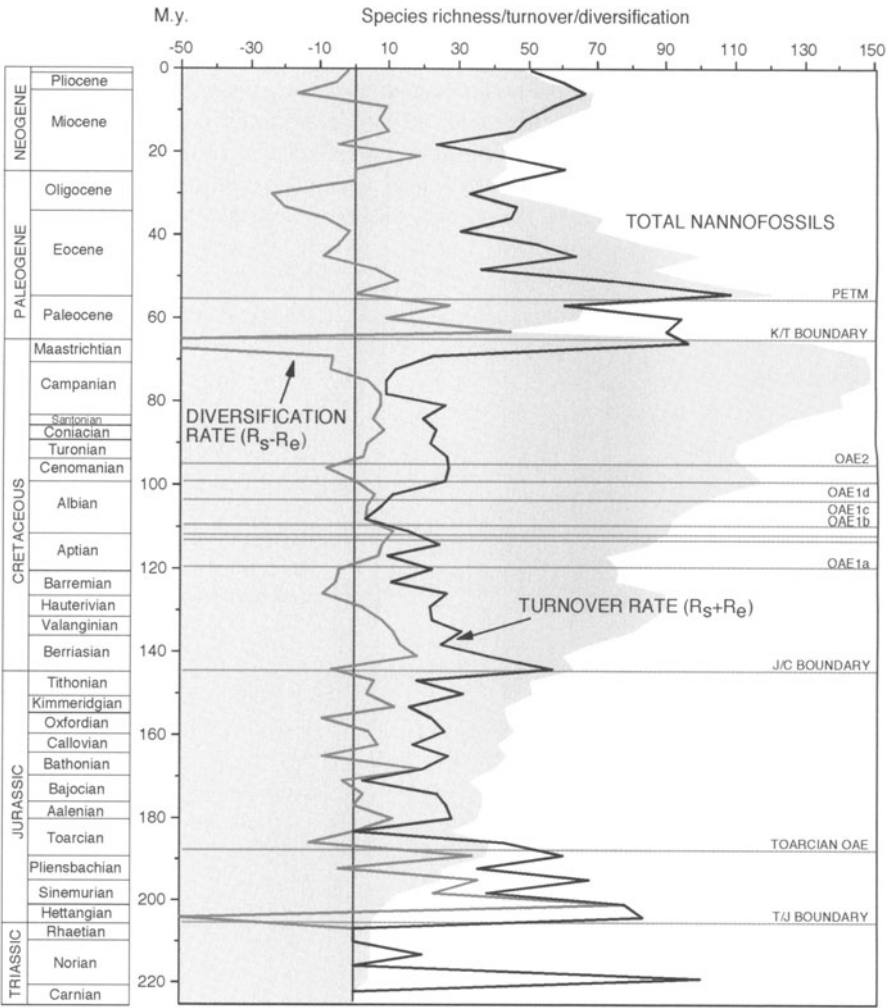


Fig. 4. Nannofossil diversity (pale shading), diversification rate (pale line) and turnover rate (dark line). R_d and R_t are measures of % change per 3 million year interval. Data plotting, time-scale and abbreviations as in Fig. 2.

the genus *Scyphosphaera* we have followed the conservative taxonomy of Young (1998) but accept that many more species have been described (around 30 Upper Miocene-Pliocene species, see synthesis of Siesser 1998); however many of these could be considered synonyms, especially when compared to the intraspecific variation displayed on extant coccospheres. Holococcolith taxa are not included, as these have very sporadic fossil records and are likely to represent life-cycle stages which have a corresponding heterococcolith stage (Cros et al. 2000; Billard

and Inouye this volume); their inclusion would thus incorrectly enhance standing diversity.

Diversity is expressed as total species present (species richness) per three million year interval, from 225 million years ago to present day. This interval duration is determined by the stratigraphic resolution at which much of the data was collected but is considered high-resolution enough to reveal real and significant patterns of taxic change (see discussion in Danelian and Johnson 2001). The data were plotted at the mid-point of the time-interval, as estimated from the time-scales of Berggren et al. (1995) and Gradstein et al. (1995) (Fig. 2). We also calculated species richness at Mesozoic substage, and Cenozoic nannofossil biozone, resolution but the trends are nearly identical and they are not included here. Similarly, generic- and family-level diversity trends are closely comparable with those shown by the species data. Both raw counts of numbers of originations and extinctions per stratigraphic interval, and established metrics of evolutionary rates, were calculated. Rates of speciation and extinction were calculated as follows: rate of speciation (R_s) = $(1/D)(FO/t)100$, rate of extinction (R_e) = $(1/D)(LO/t)100$, rate of diversification (R_d) = $(R_s - R_e)$, and rate of turnover (R_t) = $(R_s + R_e)$ (after Roth 1987), where D is total species richness, FO is number of first occurrences, LO is number of last occurrences, and t is the three million-year time-interval.

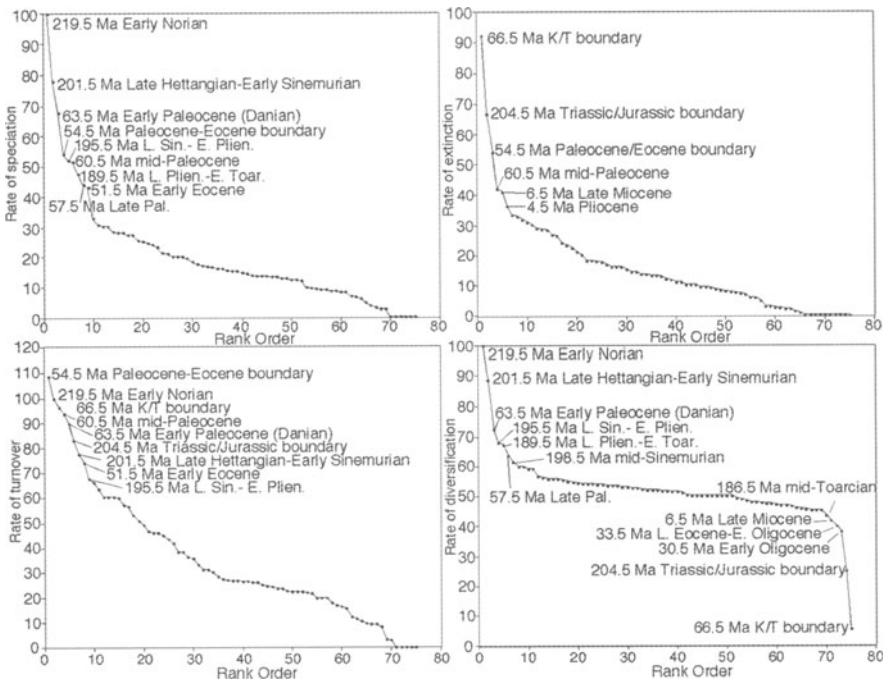


Fig. 5. Nannofossil evolutionary rates: rate of speciation, rate of extinction, rate of turnover, rate of diversification, ranked by decreasing magnitude. Above-background intervals are identified.

Because the time-interval is constant throughout this data-set, the rates have not been divided by three, and R_s and R_e are thus percent increase and decrease (Figs 3 and 4).

The evolutionary rates were plotted as ranked data-sets in order to assess the range of values present and to determine whether there are values which stand out above background (Fig. 5).

The diversity data for a number of separate nannoplankton groups have also been plotted. For the Cenozoic, Discoasteraceae, Coccolithales and Isochrysidales (synonym Prinsiales) were chosen in order to represent a range of ecological strategies, from oligotrophic- (Discoasters) to eutrophic-adapted (Isochrysidales) (Aubry 1992; Chepstow-Lusty et al. 1992; Chapman and Chepstow-Lusty 1997) (Fig. 6). In the Mesozoic, where nannoplankton paleoecologies have yet to be clearly identified, diversity was compiled for taxa which show a degree of biogeographical restriction (Fig. 7).

Quality of nannoplankton diversity data

Despite our contention that nannoplankton have a fossil record of outstanding quality, there are a number of provisos which must be considered when using and interpreting diversity data per se, and specific issues relating to nannoplankton data in particular, e.g. selective preservation, polymorphism and cryptic species.

While it may be argued that diversity patterns simply represent stochastic trends in time, with no predominant controlling mechanism, they have nevertheless proven to be extremely useful in identifying periods of significant global change, e.g. many stage boundaries, mass extinction events, etc., and there is strong support for the robustness and biological reality of diversity data at different scales and taxonomic levels (Miller 2000). The significance of the nannoplankton data is supported by the presence of parallel trends in taxonomically disparate protistan plankton groups, such as dinoflagellates and planktic foraminifera (Fig. 8), which, in turn, suggests the possibility of an overarching abiotic control which is responsible for such changes. In addition, the strength of the nannoplankton data is demonstrated by the consistency at which the main trends have been identified by studies spanning three decades, despite great changes in classification concepts and the introduction of significant numbers of new taxa. Time series diversity data for calcareous nannoplankton have previously been presented by Tappan and Loeblich (1973 – low resolution), Lipps (1970 – low resolution), Perch-Nielsen (1986 – Cretaceous and Cenozoic), Roth (1986, 1987, 1989 – Jurassic and Cretaceous), Knoll (1989 – Upper Cretaceous to Oligocene), and Bown et al. (1991, 1992 – Mesozoic to Cenozoic). Most of these studies are relatively low in stratigraphic resolution, using substages or nannofossil biozones as sample intervals.

Other, non-biological explanations for apparent parallel diversity trends have been discussed widely in the literature and include age-related sample availability (see discussion in Miller 2000) and sea-level and environment-related sampling

and preservational biases (e.g. MacLeod et al. 1997; Gale et al. 2000). These arguments do not generally apply to nannoplankton records, as discussed above, as the data is broadly global in distribution, near-continuous stratigraphically, and from across the range of marine environments.

However, there are a number of potential preservational biases which are particularly relevant to nannoplankton, namely those introduced by size and life-cycle coccolith poly- or dimorphism. Only a small proportion of modern nannoplankton are large enough, or robustly calcified enough, to be routinely preserved or observable in the geological record (Young et al. 1994). This results in a significant disparity between Quaternary diversity values (49 species) and diversity estimates for extant taxa (~220 species). A proportion of this disparity is accounted for by holococcolith diversity (~66 morphospecies), almost all of which are less than 3 μm long and none of which have a consistent fossil record, both because of their small size and delicacy. Holococcoliths do become consistent components of nannofossil assemblages in the Upper Cretaceous and, to a lesser extent, in the

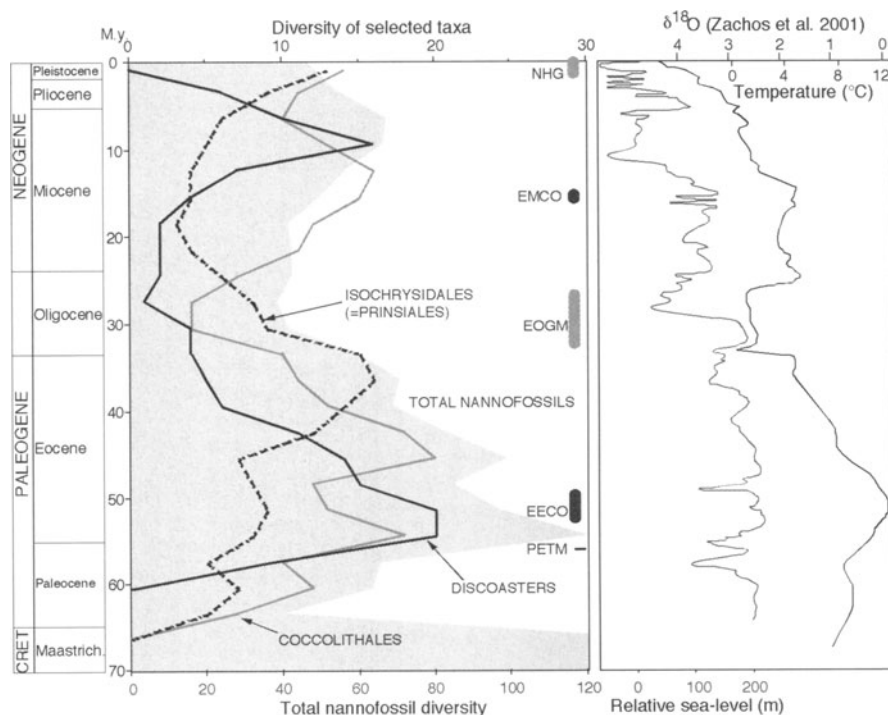


Fig. 6. Cenozoic nannofossil diversity (pale shading) with diversity for the Discoasteraceae (dark line), Isochrysidales (dashed line) and Coccolithales (pale line). Deep-sea oxygen isotope record (dark line) after Zachos et al. (2001). Relative sea-level curve (pale line) after Haq et al. (1987). PETM: Paleocene/Eocene thermal maximum, EECO: Early Eocene climatic optimum, EOGM: Early Oligocene glacial maximum, EMCO: Early Miocene climatic optimum, NHG: Northern Hemisphere glaciation.

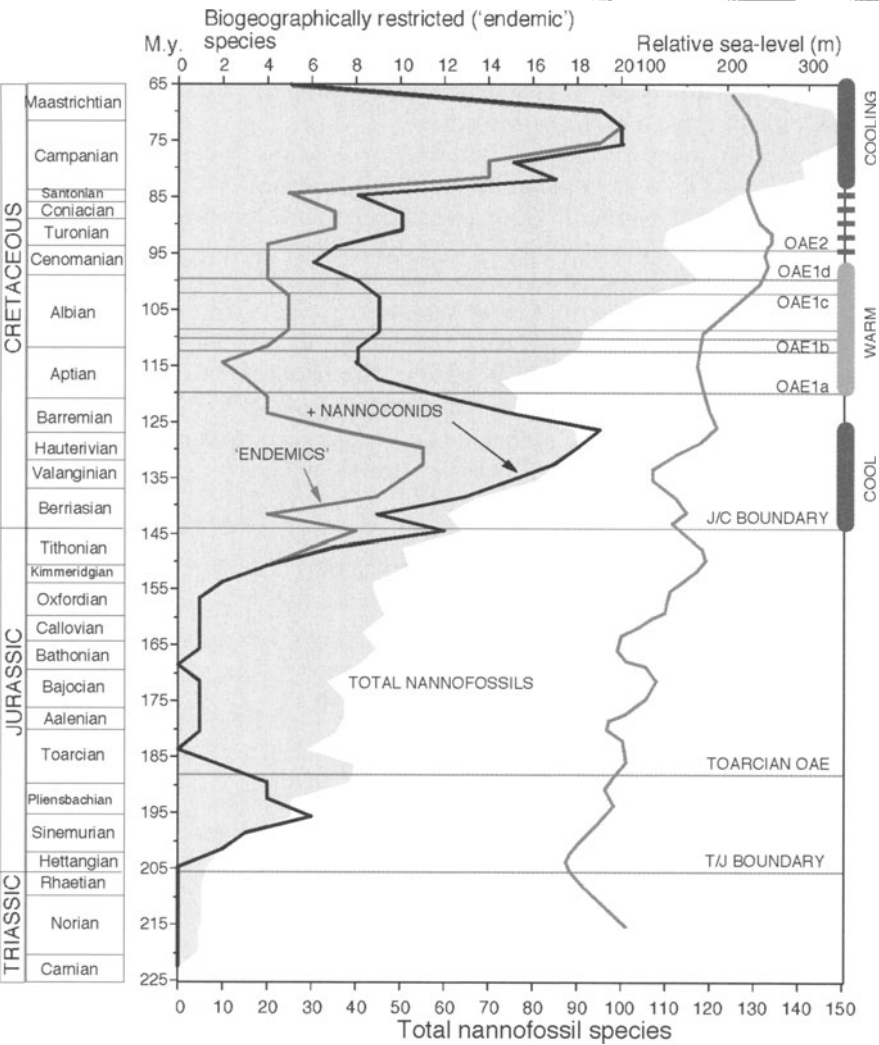


Fig. 7. Mesozoic nannofossil diversity (pale shading) with diversity for biogeographically-restricted species (pale line) and for biogeographically-restricted species plus nannoconids (dark line). Relative sea-level curve after Haq et al. (1987).

Paleogene, but these are generally larger forms. We suspect that holococcoliths have been abundant throughout the history of coccolithophores but are rarely preserved, and have avoided the potential problem by excluding them from our data, and assume that they, in any case, contribute to the diversity data via their heterococcolith counterparts.

There are also a number of living families which contribute significantly to global diversity but are only sporadically found as fossils, again due to size and

delicacy, most notably the Syracosphaeraceae (~51 extant species). However, most of the living clades have good fossilization potential, and comparison of Quaternary and extant diversities within these groups shows perfect representation. We therefore concede that not all clades may have been represented well in the fossil record, however those that are present are likely to have left a meaningful record of their evolution and diversity through time.

A qualitative survey of size distribution through time suggests at least one of the high-diversity nannoplankton episodes, in the Campanian (Upper Cretaceous), broadly coincides with increases in general coccolith size, however this relationship is not obvious elsewhere on the diversity curve.

Finally, the rapid progress in genetic characterization of extant plankton taxa has revealed the existence of cryptic species in both coccolithophore and planktic foraminifera, and this phenomenon has clear implications for diversity records. The coccolithophore 'species' *Emiliania huxleyi*, *Calcidiscus leptoporus* and *Coccolithus pelagicus* are currently the best understood in terms of genetics, coccolith biometrics and life-cycle, and may include four, three and two cryptic species, respectively (de Vargas et al. this volume; Geisen et al. this volume; Quinn et al. this volume). However, these species have been classified relatively conservatively by algologists and this inclination has been followed by those dealing with their fossil counterparts. In contrast, much paleontological nanno-

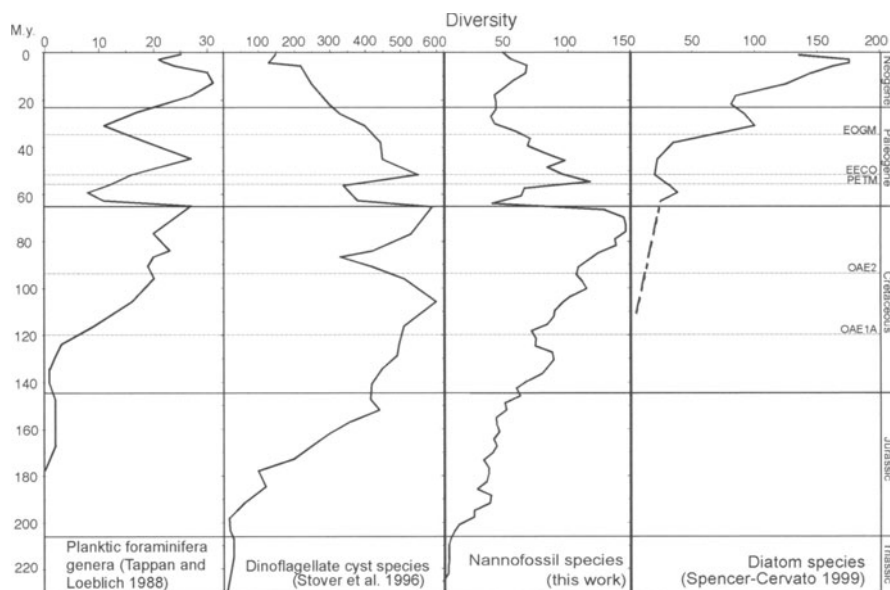


Fig. 8. Diversity of planktic foraminifera genera (after Tappan and Loeblich 1988), dinoflagellate cyst species (after Stover et al. 1996), nannofossil species (this work) and marine diatom species (Spencer-Cervato 1999). The data are collected at different stratigraphic resolutions but nevertheless provide some indication of trends in diversity between the different plankton groups. Abbreviations as in Figs 2 and 6.

plankton taxonomy has erred on the side of 'oversplitting', often subdividing similar nannofossils based on fine-scale morphological differences, in many cases driven by the desire to enhance biostratigraphic resolution. The finely-divided paleontological taxonomy is thus supported by observations of extant cryptic species and may not be greatly underestimating diversity in the fossil record, and while absolute numbers may be affected, the trends may well be the same.

Major events in nannoplankton evolution

By means of introduction to the evolution of nannoplankton, we present a brief review of the major events in their history.

225 Ma First appearance

Calcareous nannofossils first occur in the fossil record in Carnian sediments from the Southern Alps, Italy (Janofske 1992; Bown 1998b). These earliest forms are nannoliths and calcareous dinoflagellates. Coccoliths and non-dinoflagellate nannoliths are present from the Norian, but Triassic assemblages, while reasonably abundant, are of low diversity (maximum five species) and were possibly restricted to low latitudes (Bown 1998b). Triassic coccoliths are simple murolith morphologies (i.e. having narrow, wall-like rims) of very small size (2–3 μm) and show clear V/R-mode biomineralisation style.

Claims for pre-Triassic calcareous nannofossils are unsubstantiated and usually based on misidentification of inorganic calcareous objects or clear cases of contamination (reviewed in Bown 1987).

205.7 Ma Triassic/Jurassic boundary extinctions

Continuous, nannofossil-bearing boundary sections have not yet been documented, but there is strong evidence that four of the five Late Triassic nannofossil species became extinct at, or close to, the boundary (Bown 1998b). This coincides with a mass extinction event documented in invertebrate and vertebrate faunas (Hallam and Wignall 1997). One coccolith species, *Crucirhabdus primulus*, survived. Recovery following the extinctions appears to have been rather slow, although this may also reflect the paucity of productive Early Hettangian sections.

205–188 Ma Early Jurassic evolutionary radiation

The radiation of coccolithophores following the Triassic/Jurassic extinctions is one of the most important diversification events in the group's history. In just over 10 million years, nine out of a total of 16 Mesozoic families originated, repre-

senting a significant proportion of the Mesozoic and Cenozoic taxic and morphologic diversity. Coccoliths became the dominant nannofossil group, and coccolith size increased significantly. The radiation can be viewed as two separate episodes: the first saw re-establishment and radiation of the surviving Triassic coccolith group (muroliths) during the Late Hettangian–Sinemurian; the second, the appearance and radiation of new and innovative coccolith morphologies (notably placoliths) in the Pliensbachian–Early Toarcian. Holococcoliths are also first recorded at this time, but may be missing from earlier records due to preservation bias (Bown 1993).

146–140 Ma Jurassic/Cretaceous boundary turnover

The Tithonian–Berriasian saw a turnover of coccoliths at species level (17 extinctions, 15 originations) and the appearance of three important nannolith families: the Nannoconaceae, Braarudosphaeraceae and Microrhabdulaceae (see Fig. 1). It is also an interval often cited as recording the first occurrence of nannofossil-generated pelagic carbonates, an observation based on the switch from radiolaria-dominated siliceous sediments to nannofossil-dominated carbonates (Maiolica Formation) in western Tethys (Baumgartner 1987). However, earlier nannofossil-carbonates are not uncommon, e.g. the stone bands of the Kimmeridge Clay Formation, UK (Gallois 1976; Gallois and Medd 1979), and the earliest oceanic sediments, of Bathonian–Callovian age, are rather rich in calcareous nannofossils, described as marly limestones and claystones with up to 75% nannofossils (Sheridan et al. 1983). The significance of this interval in terms of nannofossil-carbonate sedimentation is, therefore, yet to be rigorously analyzed, as is the hypothesis that nannoplankton originated in shelf environments and only later migrated into oceanic habitats (e.g. Roth 1986).

80–68 Ma mid-Campanian–mid-Maastrichtian diversity maximum

The Campanian supported the highest global diversity (149 species) of fossilized nannoplankton in the evolutionary history of the group. This maximum coincided with large coccolith size and widespread distribution of nannofossil chalks.

65 Ma Cretaceous/Tertiary extinction event

The catastrophic, instantaneous and synchronous extinction of nannoplankton at the K/T boundary was a unique event in the group's evolutionary history. Of 131 Late Maastrichtian species, only nine survived: a species extinction rate of 93%, a generic extinction rate of 85%. Moreover, none of the nine survivors were common members of Late Cretaceous assemblages.

65–53 Ma Paleocene recovery and radiation

Post-K/T-extinction nannoplankton assemblages are characterized by high dominance of species which are traditionally thought of as survivor-opportunists. Interestingly, in a re-run of the Late Triassic nannofloras, small calcispheres dominated alongside the survivor coccoliths and nannoliths, and incoming Cenozoic coccoliths were initially diminutive ($\sim 2 \mu\text{m}$). New Cenozoic taxa appeared rapidly and were dominant in some areas within 50 kyr (Pospischal 1996). The evolutionary radiation that followed was both rapid and morphologically diverse. The new coccolith morphologies differed significantly from the Mesozoic architectures, and the later Paleocene was also characterized by nannolith groups (*Sphenolithus*, *Fasciculithus*, *Heliolithus*, *Discoaster*) whose radial structure almost certainly arose from coccolithophore ancestors. Evolutionary relationships between survivor lineages and new Cenozoic taxa have been conceived, but are somewhat inconclusive, largely because of problems associated with the observation of small coccoliths (Romein 1979; Perch-Nielsen 1985b; MacLeod et al. 1997).

The Paleocene radiation saw 62 species added in 10 my, compared with 32 species added in 13 my during the initial Early Jurassic radiation.

29–26 Ma Oligocene diversity minimum

Following a diversity maximum of 120 species in the Late Paleocene–Early Eocene, diversity declined rapidly through the Middle to Late Eocene, to a minimum of 39 species (10 species at nannofossil zone level) in the Oligocene. Extinctions occurred throughout the familial diversity of the group but were near-terminal in the Coccolithaceae (two species) and Discoasteraceae (two species).

11–0 Ma Miocene recovery and late Neogene decline

Nannoplankton diversity rallied in the Late Miocene (11–5 Ma), with diversification particularly in the Discoasteraceae and Ceratolithaceae. However, the trend through the Pliocene and Quaternary has been one of diversity loss.

Nannoplankton diversity patterns

The diversity plot in Fig. 2 shows data for both coccoliths and total nannofossils, and the two records show closely parallel trends. These data support the assertion that most nannolith taxa are either non-coccolith morphologies produced by haptophytes, as seen in the extant genus *Ceratolithus* (Sprengel and Young 2000), or objects produced by biologically distinct, but ecologically similar, phytoplankton groups. The larger discrepancy between coccoliths and nannofossils in the Eocene

is due to high diversities in the Discoasteraceae and Braarudosphaeraceae, and both may be coccolithophores.

Rates of speciation, extinction, diversification and turnover were plotted as ranked data-sets in order to assess the range of values present and, particularly, to determine whether there are values which stand out above background (Fig. 5). For all four metrics there is a discontinuous distribution, and a clear distinction can be made between the majority of values, which broadly fall on line and represent background rates, and more extreme values, which represent above-background events.

The Mesozoic diversity record is characterized by a dominant trajectory of increasing diversity (Fig. 2). Above-background rates of speciation ($R_s > 35$) were restricted to the Late Triassic and Early Jurassic, during the first appearance and primary radiation of nannoplankton, respectively (Fig. 3). Above-background extinction rates ($R_e > 35$) were confined to the Triassic/Jurassic and K/T boundary intervals. Speciation and extinction rates are relatively uniform through the remainder of the Jurassic and the Cretaceous, but are slightly higher at the Jurassic/Cretaceous boundary, producing a peak in rate of turnover (Fig. 4). The Cretaceous diversity increase is interrupted by minima in the Barremian–Early Aptian and Cenomanian–Turonian, and a diversity fall into the Maastrichtian. The timing of taxonomic innovation is best shown in the phylogenetic trees (Fig. 1) and, as previously discussed, clustering of suprageneric-level change is restricted to the Early Jurassic and Jurassic/Cretaceous boundary intervals.

Cenozoic diversity patterns are markedly more variable than those of the Mesozoic, and rates of speciation and extinction are consistently higher throughout the interval (Figs 2, 3). These elevated rates are particularly clear in the turnover data (Fig. 4). The Paleocene evolutionary radiation was initiated soon after the K/T boundary extinctions and proceeded rapidly, with the majority of taxonomic innovation introduced within 10 my. Diversity close to that of the Campanian Mesozoic maximum was achieved by the Early Eocene (120 species) but this was followed by rapid diversity decline to a minimum in the Oligocene (39 species). Diversity rose again through the Miocene but was followed by decline through the Pliocene and Quaternary, and the Neogene in general was characterized by lower standing diversities. Above-background evolutionary rates occurred through much of the early Paleogene, and high extinction rates were recorded in the late Neogene. The introduction of major taxonomic innovation was largely restricted to the Paleocene radiation.

The taxon-specific diversity data reveal patterns that are clearly distinct from global diversity. The Mesozoic biogeographically-restricted taxa display strong diversity peaks in the Early Jurassic, Early Cretaceous (Berriasian–Barremian) and Campanian (Fig. 7). The main Cenozoic nannofossil groups each show somewhat similar trends, with diversity peaks in the Paleogene and Neogene, separated by Oligocene minima, but there is a consistent offset between each record (Fig. 6). The Discoasteraceae show peaks in the Early Eocene and Late Miocene, and near-extinction and extinction in the Oligocene and latest Pliocene, respectively. The Coccolithales show peaks in the Middle Eocene, Middle Miocene and Late Neogene. The Isochrysidales peak in the Late Eocene and Late Neogene.

Explaining diversity patterns in the fossil record

Diversity patterns in the fossil record have been attributed to a raft of causal factors, ranging from biotic causes such as competition and ecological replacement to, more commonly, abiotic causes such as environmental stability, climate change, sea-level variation (and consequent area effects), plate tectonic configurations and nutrient supply or productivity of oceanic water-masses. In reality, these factors may all play a role in controlling global diversity and many of them are inexorably interrelated. Two models in particular have been championed in recent years. In simple terms, the energy-bound model of Vermeij (1995) suggests that increasing nutrient supply results in diversification, as population growth-rates increase and ecological constraints on innovation decrease. Conversely, Hallock (1987) argues that the nutrient-controlled expansion and contraction of a continuum of oceanic trophic resources leads to diversification during intervals of reduced nutrient supply and extinctions during periods of increased nutrients. Observations on the biogeography and ecology of extant nannoplankton (McIntyre and Bé 1967; Okada and Honjo 1973; Winter et al. 1994; Thierstein et al. this volume) suggest that the Hallock model best explains present day nannoplankton diversity distribution. Extant coccolithophore diversity is strongly influenced by high-diversity but low standing-crop, K-mode-dominated communities living in stable, oligotrophic, subtropical ocean-gyre water-masses. Areas of high, and usually pulsed, nutrient delivery (high latitude and upwelling sites) support low-diversity, high standing-stock populations dominated by one or more r-mode taxa and, in extreme cases, monospecific blooms. The diversity patterns of fossil nannoplankton can thus be interpreted by analogy with their living counterparts: increased diversity reflecting expansion of oligotrophic habitats, and extinctions caused by reduction of oligotrophic habitats and expansion of eutrophic areas.

Mesozoic nannoplankton evolution and diversity

The Mesozoic diversity data is plotted against sea-level and a limited number of paleoclimate observations (Fig. 7). Broad inferences can be drawn concerning the Mesozoic climate-ocean system: for example, it is thought to have been characterized by globally-warm greenhouse conditions with little or no polar ice and low pole-to-Equator thermal gradients. However, more detailed records of change within this framework remain enigmatic (see reviews by Frakes et al. 1992; Frakes 1999; Gale 2000; Burnett et al. 2000). Most workers now agree that the mid-Cretaceous supported climates of exceptional warmth and that this was followed by Late Cretaceous cooling which varied in structure and timing with latitude. The isotope records of Jenkyns et al. (1994 – English Chalk) suggest that long-term cooling was initiated in the Turonian, whereas those of Huber (1998 – Southern Ocean) support continued high-latitude warming in the Early Turonian and sustained warmth into the earliest Campanian, followed by long-term cooling through the Maastrichtian.

The broadly-increasing diversity pattern of the Mesozoic (excluding Triassic/Jurassic boundary extinctions) is suggestive of long-term stability and widespread oligotrophy in photic-zone environments. This supports the generalized inferences described above but does not preclude the existence of fluctuations in the Jurassic–Cretaceous ocean-climate system, rather indicating that such fluctuations were not as great, rapid or sustained as those of the Cenozoic.

The appearance of nannoplankton in the Late Triassic, and the near-coincident appearance of dinoflagellates, has been attributed to evolutionary opportunity following ecosystem clearance during the end-Permian mass extinction (Bown et al. 1991). Nevertheless, the time-lag between the mass extinction and the appearance of nannofossils is almost 30 my. Molecular analysis shows that the two phytoplankton groups have much longer histories, probably stretching back to the Proterozoic (Medlin et al. 1997). The appearance of coccoliths in the Triassic is therefore almost certainly a biomineralisation innovation, but possibly linked with a secondary symbiotic event.

The initial evolutionary radiation of nannoplankton was interrupted by extinctions at the Triassic/Jurassic boundary, which were part of a mass extinction event which has been variously related to impacts (Olsen et al. 2002), igneous activity (Wignall 2001), sea-level (Hallam and Wignall 1997) and global carbon-cycle perturbation (Hesselbo et al. 2002). This event may yet prove comparable to the K/T boundary event but the real significance of the event for nannoplankton is difficult to judge due to the low Triassic diversities involved. The survival of just one species, however, suggests it may well have been a catastrophic reduction and raises the possibility that wholesale extinction of nannoplankton may have been involved in marine ecosystem collapse in both Mesozoic mass extinctions.

The Early Jurassic evolutionary radiation was initiated following these extinctions and by the Early Toarcian most major innovations had been introduced. Consequently, speciation and diversification rates fell to levels which were broadly maintained through the remainder of the Mesozoic (background levels). This pattern of initial evolutionary experimentation followed by long-term stability is shown by many groups of organisms (Gould et al. 1987) and has been termed 'evolutionary equilibrium' (Sepkowski 1978) or 'diversity equilibrium' (Wei and Kennett 1986).

Slightly elevated rates of speciation, extinction and turnover occurred around the Jurassic/Cretaceous boundary, and species-level turnover was high, as discussed above. This interval is associated with substantial faunal changes (hence the position of the system boundary) but is rather poorly understood in terms of environmental change. High rates of diversification in radiolaria, and a shift from radiolaria- to nannofossil-dominated sedimentation, have been explained by a climate-forced reduction of nutrient delivery to the western Tethys and Atlantic Oceans in the Tithonian (Danielian and Johnson 2001). This may also explain nannoplankton speciation at this time, particularly as western Tethys appears to have been a locus of evolutionary activity during the Jurassic (Bown 1987). However, the Tithonian also saw the introduction of considerable biogeographic differentiation, and diversification within separate nannofloral provinces may well have significantly contributed to global biodiversity.

Cretaceous nannoplankton evolutionary history was characterized by overall diversity increase but bracketed by more dramatic events at the base and top of the period. Diversity rise was not uniform, rather three intervals of increase were interrupted by minima spanning the Barremian–Early Aptian and Early Cenomanian–Turonian, and a slight fall into the Maastrichtian.

The Berriasian–Barremian and Turonian–Campanian periods of diversity increase broadly correlate with putative cooler climate intervals (Huber 1998; Frakes 1999), and the Campanian diversity maximum may have been related to the onset of late Mesozoic high-latitude cooling. Both intervals are clearly accompanied by significant peaks in diversity of biogeographically-restricted taxa at both high and low latitudes, and this is particularly clear in the Campanian, with the development of a well-defined and diverse Austral province (Watkins et al. 1996; Lees 2002). These observations strongly suggest that cooler Mesozoic climate intervals led to increased heterogeneity within photic zone environments, greater biogeographic partitioning, and hence diversity maxima. The mid-Cretaceous interval, considered a time of exceptional warmth, saw significantly reduced numbers of endemic taxa and this may explain the diversity minimum which preceded it; diversity nevertheless continued increasing through the Late Aptian and Albian. Similarly, biogeographic reorganization in response to climate amelioration may explain the reduction of diversity in the mid-Maastrichtian (Huber and Watkins 1992; Watkins et al. 1996; Lees 2002).

One of the most interesting features of the Cretaceous phylogenetic and diversity data is the lack of a clear relationship between nannoplankton evolution and the Oceanic Anoxic Event (OAE) intervals. These events are widely believed to represent significant regional or global perturbations of the marine environment, characterized by rapid carbon burial and incorporating aspects of extreme warmth, widespread oceanic stagnation and anoxia, and possibly enhanced primary productivity (Leckie et al. 2002), and they have been widely cited as significant events in nannoplankton history (Roth 1987, 1989; Bralower et al. 1994; Leckie et al. 2002). Our data show that rates of evolutionary change never rose above Mesozoic background levels during the OAEs, and the intervals are not characterized by significant taxonomic loss or innovation. It is notable that the global OAEs, 1a and 2, both occurred within the diversity minima noted above, but decrease occurred substantially prior to their onset. Although taxon-specific nannoplankton abundance fluctuations are associated with OAE1a (e.g. Erba 1994), and links between planktic foraminifera evolution and OAEs appear to be strong (Leckie et al. 2002), there is no case for major turnovers in the case of calcareous nannoplankton.

Nannoplankton, more than any other fossil group, have informed our detailed understanding of the timing, magnitude, and geography of extinctions at the K/T boundary, and subsequent recovery and radiation. Worldwide, high-resolution studies have consistently reported rapid (i.e. geologically instantaneous), synchronous, near-terminal extinctions across the biodiversity of the nannoplankton group, accompanied by a collateral crash in pelagic carbonate sedimentation (Thierstein 1981; Pospichal 1994, 1996; Gartner 1996; MacLeod et al. 1997; Burnett 1998). Our data corroborates previous observations, with nine out of 14

coccolithophore families terminated at the boundary and 93% extinction rate at species level. Recovery following the extinctions was rapid, and the rather atypical survivor groups (see MacLeod et al. 1997) were soon joined by new Cenozoic taxa whose origins were rather cryptic.

Cenozoic nannoplankton evolution and diversity

The Cenozoic nannoplankton data is plotted against paleoclimatological information, including long-term climate trends and intervals of particular interest, e.g. the Paleocene/Eocene Thermal Maximum, Early Eocene climatic optimum, etc. (Fig. 6). Broadly, there is good correlation between nannoplankton diversity and climate trends: diversity generally increased during periods of climate warming (e.g. Paleocene–Early Eocene, Miocene) and decreased as climate cooled (e.g. Middle Eocene–Oligocene, Pliocene–Recent). The Paleocene radiation occurred during a period of warm greenhouse climate, probably comparable to that of the late Mesozoic. The Cenozoic diversity maximum in the Late Paleocene–Early Eocene corresponded to a climatic optimum and maximum dispersal of warm-water taxa (Pospichal and Wise 1990). Diversity decline through the Eocene closely parallels climatic deterioration marked by falling surface- and deep-water temperatures (Zachos et al. 1994, 2001). The Cenozoic diversity minimum occurred during the Oligocene glacial maximum, which saw the initial establishment of significant Antarctic glaciation. We acknowledge that these correlations are of low resolution, however, they appear to be significant events in terms of long-term change, especially since parallel trends are seen in other plankton groups (see below).

The correlation between diversity and climate is particularly striking in the Paleogene, but the relationship contrasts with the Mesozoic observations, described above. This suggests that the magnitude of climate cooling in the Eocene did not support diversification through biogeographic partitioning but rather reduction of diversity due to the contraction of stable oligotrophic habitats and the suppression of diversity at temperate and high latitudes: high diversity at high latitudes was a feature of the Mesozoic. This interpretation is further supported by the taxon-specific diversity records, and particularly the diversity history of the warm-water, oligotrophic-adapted Discoasteraceae. Maximum discoaster diversity coincided with the group maximum and then declined precipitously through the Eocene to near-extinction in the Oligocene. The mesotrophic-adapted Coccolithales peaked in diversity in the Middle Eocene, coinciding with a short recovery in group diversity (the Cenozoic maximum at nannofossil zone resolution), and echoing Mesozoic observations of diversity increase accompanying cooling. The cooler-water, meso-eutrophic-adapted Prinsiales increased in diversity through the Eocene but declined sharply in the Oligocene. The records of these taxa reveal contrasting responses to climate change which can be explained by their different ecological strategies, but underlines the importance of the K-selected groups, in this case discoasters, in terms of global diversity.

These global and taxon-specific diversity records are remarkably similar to patterns observed in planktic foraminifera, which show declining diversity but successive peaks in warm-, temperate- and cool-adapted groups through the Eocene (Boersma and Premoli Silva 1991; Hallock et al. 1991). These authors also observed high Middle Eocene diversity, attributing it to the coexistence of older, 'warm-world' biotas with a new, 'cooling-world' biota, describing this as a temporal or evolutionary ecotone (Hallock et al. 1991), an observation which is also applicable to the nannoplankton record.

The similar evolutionary histories of these two biologically and ecologically distinct plankton groups is strong evidence of abiotic, and specifically climatic, control on their evolution at this time. However, it should be noted that the marked decline in nannoplankton diversity in the Late Eocene and Oligocene was accompanied by exponential rise in marine diatom diversity (Spencer-Cervato 1999), although this may well reflect an expansion of habitats conducive to diatom diversification, i.e. cool high-latitude water-masses, rather than direct competition between the two phytoplankton groups (Fig. 8).

These diversity patterns conform to the Hallock hypothesis, with K-selected taxa proliferating during warming and warm intervals, as stable oligotrophic habitats and the trophic resource continuum expanded, creating more potential habitat space, and the increased importance of meso- and eutrophic-adapted groups during periods of cooling.

The Neogene record is less straightforward. Nannoplankton diversity recovered through the Middle to Late Miocene, and the Discoasteraceae underwent a renewed period of radiation, culminating in the Late Miocene diversity maximum. This is suggestive of an expansion of warm, stable oligotrophic habitats during the Miocene but does not correlate particularly well with our current understanding of Miocene climates and oceans, coming soon after a significant cooling event in the deep-water oxygen isotope record (Zachos et al. 2001). However, oxygen isotope records from planktic foraminifera suggest that, while cooling proceeded relatively rapidly at high latitudes (and ice built up on Antarctica), surface-water temperatures were stable, or even warmed, in temperate, subtropical and tropical regions (Savin et al. 1985).

As in the Eocene, the Isochrysidales showed diversity increase through the Pliocene and Pleistocene, against a general background of declining diversities. The terminal decline of discoasters occurred during a time of cooling and onset of glaciation in the Northern Hemisphere, however, the correlation is not exact, and in fact extinctions of the final species, *Discoaster brouweri* and *D. triradiatus*, are recorded in an interglacial (Chapman and Chepstow-Lusty 1997).

High rates of speciation and extinction throughout the Cenozoic suggest that a level of evolutionary stability ('equilibrium') was not attained in the last 65 my. High speciation and turnover rates were initially associated with the post-K/T recovery and radiation, but the high extinction rates, which are recorded relatively consistently, appear to be associated first with the Paleocene/Eocene boundary event, and second the Eocene–Oligocene climatic deterioration associated with the onset of Cenozoic glaciations. The rapid and high-amplitude climate changes appear to have consistently maintained evolutionary rates at levels significantly

higher than those seen in the Mesozoic, and resulted in an overall pattern of diversity decline from the Eocene to present. These statistical observations are supported by qualitative comparisons of nannoplankton assemblages, with far greater differences seen between the Cenozoic epochs compared with for example, the Cretaceous, stages. Either abiotic or biotic factors forced higher turnover in the Cenozoic, or there is a fundamental difference in the nannoplankton which evolved following the K/T boundary extinctions.

Conclusion

The combined information from nannoplankton phylogenetic trees and diversity data provide us with an outstanding record of evolutionary history for this phytoplankton group. Although not all extant clades have been well represented in the fossil record, those that are present are likely to have left a representative and meaningful record of their evolution and diversity through time. The existence of cryptic species and life-cycle dimorphs (holococcoliths) may not greatly affect the diversity trends we have observed.

There is good correlation between previously-documented extinction data and the nannoplankton record, with the two major post-Paleozoic mass extinction events shown clearly by significantly above-background evolutionary rates. In fact, it is conceivable that extinction in the marine realm may have been abetted by eradication of the primary-producer phytoplankton during these events. Although the primary extinction mechanism is yet to be identified, it could possibly have been due to impact-induced darkness or hyperthermal-induced calcification crises. The two major nannoplankton evolutionary radiation events occurred directly following these extinction events.

Coccolithophores and associated nannoplankton showed a pattern of broadly increasing diversity through the Mesozoic, suggesting long-term stability and widespread oligotrophy in photic-zone environments. Jurassic/Cretaceous boundary turnover and taxonomic innovation may have been linked to reduced nutrient cycling in western Tethys and/or biogeographic differentiation, but may also reflect more profound ocean-climate changes at this time, which we have yet to identify. Two important Cretaceous periods of diversity increase (Neocomian and Turonian–Campanian) were both associated with increased levels of biogeographic differentiation, most likely linked to climate cooling. Reduced biogeographic partitioning in the mid-Cretaceous, linked to warm climates, may explain the diversity decline which preceded it. The two major Cretaceous OAEs (OAE1a and OAE2) fall within intervals of reduced diversity but are not obviously associated with above-background evolutionary rates or significant taxonomic loss or innovation.

Cenozoic diversity records are closely correlated with climate change, increasing diversity associated with climate warming and decreasing diversity with cooling. This contrasts with Mesozoic diversity patterns and indicates that the magnitudes, rates and duration of Cenozoic cooling prevented diversification through

biogeographic differentiation and, instead, saw suppression of diversity at temperate and high latitudes. Warm intervals supported high diversities of warm, K-selected taxa, such as discoasters, whereas cool and cooling climates saw increases in meso- and eutrophic-adapted groups, such as the Coccolithales and Isochrysidales (synonym Prinsiales). Miocene diversities are not well correlated with paleoclimate trends but suggest that, while cooling proceeded at high latitudes, particularly associated with ice-sheet growth in Antarctica, warmer, oligotrophic habitats were sustained or expanded at low latitudes, supporting high global diversity and the diversification of the K-selected discoasters. As bipolar glaciation was established in the late Neogene, discoasters declined to extinction, the Prinsiales group increased, but diversity decreased overall. High rates of speciation and extinction were sustained throughout the Cenozoic, and evolutionary equilibrium was never achieved. The overall pattern of diversity decline from the Eocene to present suggests that onset and evolution of the icehouse-mode climate-ocean system resulted in higher extinction rates, lowered diversity in temperate and high latitudes, and global diversity minima during the Oligocene and Pliocene–Holocene.

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Carbonate fluxes and calcareous nannoplankton

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Summary

Coccolithophores first became significant participants in the carbonate cycle in the Jurassic, but throughout the Jurassic they were largely restricted to shelf and epeiric sea environments. They spread into the open ocean in the Cretaceous, and with this became a major factor in governing the carbonate cycle in the sea. With the development of dissolution-resistant forms, such as *Watznaueria barnesae*, the coccolithophores perturbed the carbonate system and switched the major site of carbonate deposition from shallow seas to the deep ocean. Several major evolutionary steps in the development of the coccolithophores have forced further changes in the carbon cycle, favoring the deep sea as a site of carbonate deposition. Samples of recent coccolith assemblages from bottom sediments differ from those of living coccolithophores in surface waters. Many of the coccoliths of more delicate species, particularly holococcoliths, are dissolved in the water column or at the sediment surface and are only rarely preserved as fossils. They, along with the pteropods, form an important part of the shallow carbonate cycle. There appears to be a continuous gradation in the level of susceptibility of coccoliths to dissolution, from forms that dissolve in the near-saturated waters of the surface ocean to those that are among the most dissolution-resistant forms of calcite. This continuous dissolution spectrum is in contrast to the planktic foraminifera, in which dissolution of the tests also occurs in a sequence, but through a much more restricted depth range, the lysocline. Whereas the order of dissolution of planktic foraminifera follows their habitat, with warm-water species being most susceptible and cold-water forms most resistant to dissolution, the order of dissolution of coccoliths appears to be related to phylogeny. The steepness of the coccolith carbonate dissolution gradient appears to have changed over time. In the Oligocene almost pure nannofossil carbonate oozes devoid of terrigenous material were widespread, perhaps reflecting unusual climatic conditions on land. The overall effect of coccolithophore evolution has been to move carbonate deposition to the deep sea, where coccolith oozes accumulate on ocean crust and will ultimately be subducted. Only a fraction of the carbon in the subducted carbonate is returned to the surface through volcanic activity. If their activity were to continue for several

hundreds of millions of years the coccolithophores would remove much of the carbon from the surface of the Earth to be emplaced in the mantle.

Introduction

Calcareous nannoplankton fossils form a significant part of the modern carbonate depositional system and have been important since the Late Mesozoic. Along with the planktic foraminifera, they may be acting to change the way in which the entire sedimentary system operates. These two groups are acting to shift the primary site of deposition of calcium carbonate from the margins of the continental blocks where it can be preserved for long periods of time to the deep sea floor where subduction processes will act to recycle the calcium back into the Earth's mantle and ultimately return the carbonate to the atmosphere as CO_2 to participate in rock weathering processes. This paper will discuss the partitioning of carbon between organic carbon and carbonate, describe the role of carbonate in the sedimentary system throughout the Phanerozoic, and describe the role of the calcareous nannoplankton in modifying the sedimentary system on both long and short-term time-scales.

The data on Phanerozoic sediments

Knowledge about the temporal distribution of sedimentary materials on the Earth stems mostly from the compilations made by Alexander Borisovitch Ronov, his colleague Victor Efimovitch Khain and their co-workers Kyril Seslavinsky, Alexander Balukhovsky, and Areg Migdisov at the Vernadsky Institute of Geochemistry of the Academy of Sciences of the U.S.S.R. (now Russian Academy of Sciences) in Moscow. Started in 1947, the compilation includes data on areas, volumes and masses of sediments from all of the continents except Antarctica, and from the ocean basins. The data compilation originally recognized 13 kinds of sediment on the continental blocks and their margins (continental terrigenous sediment, coal-bearing terrigenous sediment, glacial sediment, molasse, marine sands, marine shales, marine terrigenous sediment, flysch, carbonate-terrigenous sediment (marls), carbonates, halite, gypsum/anhydrite, and siliceous sediment). The term "carbonates" includes all aragonitic, calcitic and dolomitic sediments and rocks. Additional sediment types were added for the deep sea as information from the Deep Sea Drilling Project and Ocean Drilling Program became available. A summary of the compilations of sediment on the continental blocks and on the ocean floor is shown in Fig. 1.

From the beginning of the project in the 1940's, when most geologists considered continents and ocean basins to be fixed permanent features of the earth's surface, the data were collected for regional sources and sinks at a subcontinental scale source-sink regions, but only continental (Ronov and Khain 1954, 1955,

1956, 1961, 1962; Ronov et al. 1974; Khain et al. 1975; Ronov et al. 1976, 1977; Khain et al. 1977; Ronov et al. 1978; Khain et al. 1979) and global summary compilations have been published (Ronov 1980, 1993). The Ronov database gives areas, volumes and masses of existing sediment for 28 units of the Phanerozoic and three units of the Late Proterozoic. The 28 Phanerozoic units represent two- and three-fold subdivisions of systems and the epochs of the Tertiary. Although they are millions of years long, these are still the shortest intervals that can be reliably correlated between different sedimentary environments. The published data were presented in terms of distribution of materials in three tectonic environments: platforms, geosynclines, and orogenic regions on the five major continents (Eurasia, North America, South America, Australia, Africa). "Platforms" correspond to both cratonic regions and the surrounding sediment-covered continental platforms. "Geosynclines" – a term that was widely used at the time the compilation began – are areas where unusually thick accumulations of sediments were deposited. "Orogenic regions" are areas of mountain-building activity and were major source regions for sediments although smaller amounts of material were deposited in intermontane basins within the orogenic regions.

As a means of evaluating the reliability of the compilations, Ronov (1993) compared four estimates of the total global mass of sedimentary rocks obtained using different data and methods. His estimate (113.0×10^{21} kg) coincided within 5% to that of Southam and Hay (1981; 111.5×10^{21} kg) and of Khain et al. (1982; 110.4×10^{21} kg). Only Kunin's (1987) estimate (97.0×10^{21} kg), based on extrapolation of geophysical data on Eurasia to other continents differed from other estimates, but even this discrepancy was no more 13%.

A recent test of the precision of the data collected by Ronov, Khain and their coworkers, was done by Berry and Wilkinson (1994) who compared the Ronov group's estimates of volumes of sediment with their own estimates of volumes for North America made by digitizing the Cook and Bally (1975) atlas. The Ronov group's estimates were generally about 10% higher than those made using the Cook and Bally atlas for platform areas, and consistently about 1/3 higher for the "geosynclinal" regions. The estimates are remarkably close in view of the differences in information available and methodologies. The greater values given by the Ronov group for "geosynclinal" regions have to do with the assumptions made on volumes of deformed rock. The Ronov data have been generally accepted as a standard base for global analysis of volumes and masses of sedimentary materials and are used here to explore the distribution of carbonate rock through time.

The Quaternary remains a special problem. There is no consistent method used in different areas for showing the extent of Quaternary sediments, and data on their thickness is often unavailable or incomplete. Global compilations for Early and Late Quaternary consistent with the Ronov data for older sediments are in the course of preparation by Alexander Balukhovskiy at the Vernadsky Institute for Geochemistry of the Russian Academy of Sciences in Moscow. The only global summary compilation available at present is that by Hay (1994). Since the bulk of Quaternary sediment is in the deep sea and is relatively well known, Hay's estimate is probably comparable with the global totals for the earlier time periods in the Ronov database.

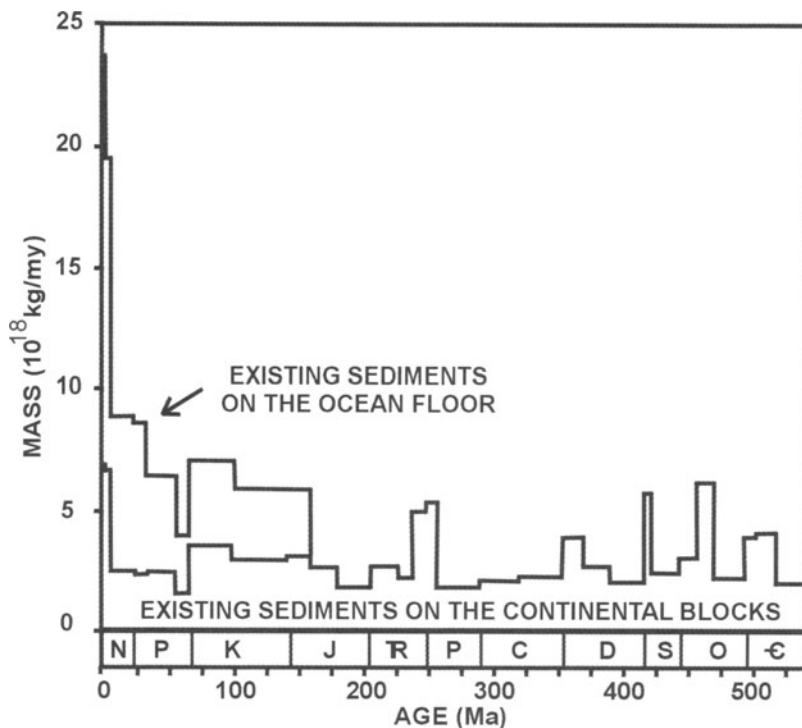


Fig. 1. Mass-age distribution of sediments, sedimentary rocks, and metasediments on the continental blocks and the additional sediment that lies on the ocean floor, based on data from Ronov (1993) and Hay (1994).

Fig. 1 shows the global mass age distribution based on Ronov (1993), adjusted to add in Antarctica under the assumption that the distribution of its sediments approximates the average for the other continents and their mass is proportional to the area of the continent. One peculiarity of the mass-age distribution is the apparent great increase in sedimentation rate during the Neogene and Quaternary, both on the ocean floor and the continental blocks. This phenomenon suggests that there has been a change in the erosion – sedimentation mechanism since the beginning of the Neogene. Hay et al. (2002) have discussed this problem and its possible causes. They suggested that the spread of C_4 plants may have altered the weathering-erosion-sedimentation system.

The problem of destruction of older sediment through erosion

Ronov and his colleagues realized that the existing sedimentary record has been affected by erosion, and that some of the older record has been destroyed. In

several publications they attempted to account for part of this effect by extrapolations from the eroded edge of existing deposits. However, this technique made only minor corrections and did not account for deposits which have been mostly or completely removed by subsequent erosion. Gilluly (1969) noted that the overall distribution of existing sedimentary rocks shows generally decreasing areas (or volumes, or masses) with age. He explained this by noting that the sedimentary system is cannibalistic – most young sediments are created from the erosion of older sediments. This being the case it becomes possible to treat the existing sedimentary record as the product of an exponential decay. It is assumed that a small proportion of young sediments is generated from the weathering of igneous and metamorphic rocks, and some old sediments are lost to metamorphism and subduction. For the terrigenous sediments carried as the suspended and traction loads of rivers, by the winds, and by glaciers, the time between erosion and deposition is usually very short, essentially geologically instantaneous, but soluble materials such as limestone and salt can be stored in the ocean for thousands to millions of years. Nevertheless, the shortest of the time units in the Ronov compilation, the Pliocene (ca. 3.5 million years long), is longer than the residence time of calcium (1.4 million years taking only the riverine flux into account, about 0.7 my taking both riverine and hydrothermal fluxes into account) and bicarbonate (0.1 my) in the ocean. On the time-scale of the Ronov compilation the erosion and deposition of carbonate can also be considered to be instantaneous. Thus, on the long-term time-scales over which the data can be analyzed, what goes in must come out – erosion and sedimentation of terrigenous materials and carbonates are “instantaneous” in that they occur within the same interval.

Wold and Hay (1990) presented a simple method for reconstructing the original masses of sedimentary rock deposited based on the assumption that variations from a long term decay of the sedimentary mass with age are real and reflect variations of the erosion-deposition rates with time. The method involves fitting an exponential decay curve to the data. Reconstruction of the original amount of sediment deposited is accomplished by multiplying the proportional excess or deficit of existing sediment for each age relative to the decay curve by the y-axis intercept of the decay curve. It assumes that variations of the existing sediment masses relative to the long-term exponential decay represent real changes in erosion-sedimentation rates at each time interval. This technique was used to reconstruct the originally deposited sediment masses shown in Fig. 2.

Wold and Hay (1990) argued that these variations must reflect primarily orogenic activity and to a lesser extent climate change. McArthur et al. (2001) and Hay et al. (2001) have noted that there is a good correlation of the variations in erosion and deposition rates and the marine Sr-isotope curve for the Phanerozoic. This would seem to support the hypothesis that the variations are primarily a reflection of orogenic activity. However, Berner (1997) has recently discussed the effects of the spread of land plants in the Silurian–Devonian, and it seems likely that the great increase in erosion-deposition at that time may be in large part the result of changes in weathering rates and soil formation as land became covered with plants. It is important to note that the reconstruction shown in Fig. 2 indicates

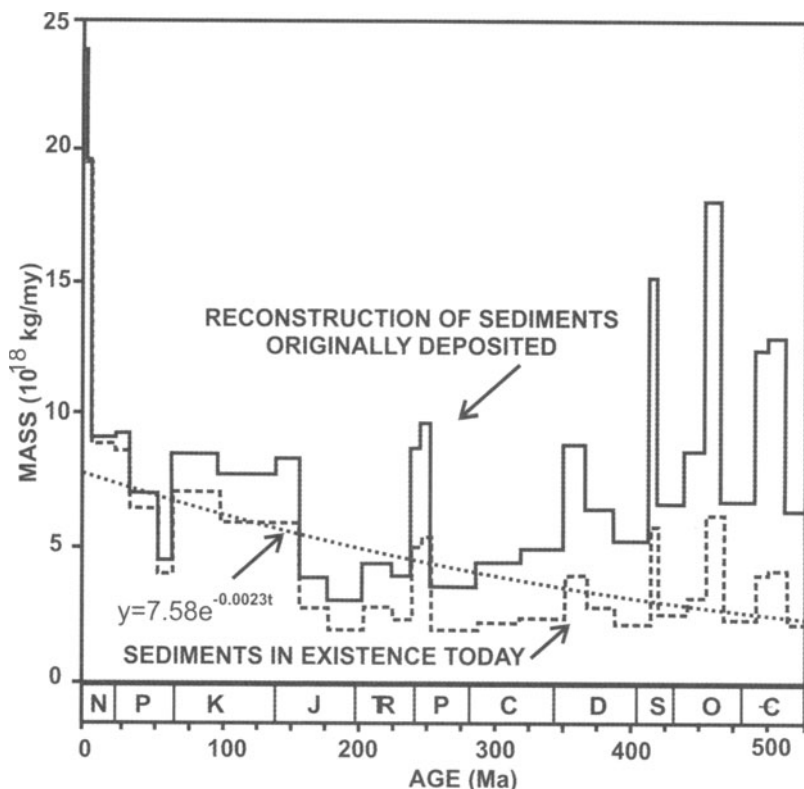


Fig. 2. Reconstructed fluxes of sediment during the Phanerozoic. The exponential decay curve (dotted) is fit to the Phanerozoic data from Ronov (1993) and the Quaternary estimate of Hay (1994). The solid line is the reconstruction based on the assumption that the total sedimentary mass has remained constant. The dashed line represents the present mass-age distribution of sediment.

that there have been times in the past, particularly during the early Paleozoic, when erosion-sedimentation rates similar to those of the Neogene-Quaternary have prevailed. These high rates occurred mostly before the spread of land plants, when weathering, soil formation, and erosion processes were very different from today.

Wold and Hay (1993) noted that the method described in 1990 does not result in a constant sedimentary mass, and showed how the constraint of a constant sedimentary mass or a defined rate of increase or decrease in the sedimentary mass with time could be applied. They also argued that although the rates of sedimentary recycling may change with time, all sediment types must recycle at the same rate within a given time interval. The argument rests on the fact that sedimentary layers are very thin compared with their lateral extent, and that particular lithologies cannot be “mined” from beneath the overlying layers. The only significant exception might be soluble evaporite deposits and particularly halite which

may ascend as diapirs from their original site of deposition. However, even in the case of evaporites it is unlikely that significant erosion could occur before the removal of most of the overlying strata, and on the time-scale available for the Ronov data set this problem has been considered to be insignificant (Floegel et al. 2000; Hay et al. 2001).

The partitioning of carbon between organic carbon and carbonates

Carbon may be sedimented as carbonate or as organic carbon. In modern plankton, more than four moles of C are fixed as C_{org} for every mole fixed as $CaCO_3$. As these are incorporated into sediment, the relative proportions are reversed: at present and during the late Cenozoic about six moles of C are buried as $CaCO_3$ for every mole buried as C_{org} . Fig. 3 shows the ratio of C sedimented as $CaCO_3$ to C deposited in sediments as organic carbon, based on data in Ronov (1993). Times of major organic carbon deposition as either coal or marine shales rich in C_{org} have

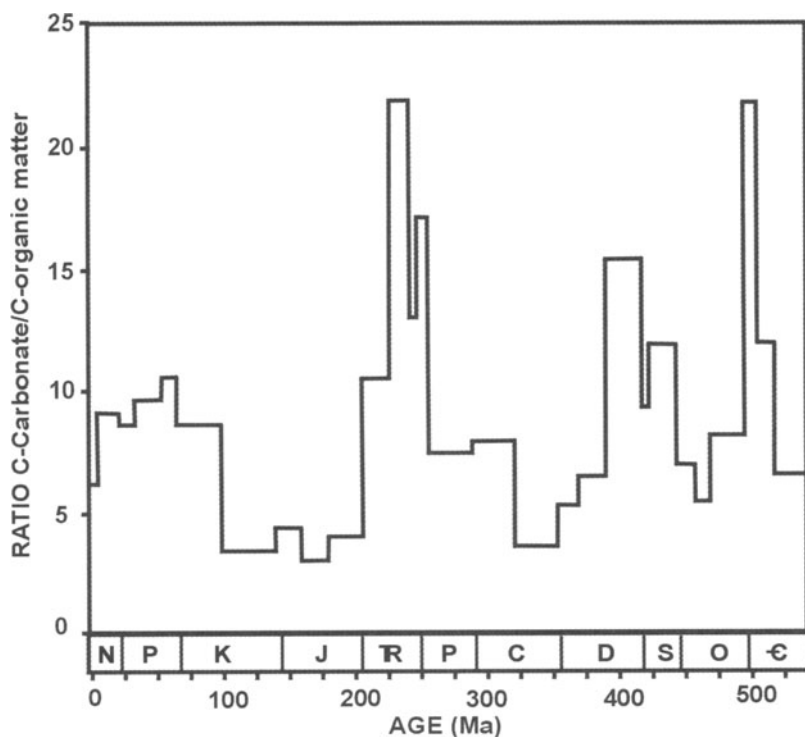


Fig. 3. The ratio of carbon deposited as carbonate to carbon deposited as organic carbon during the Phanerozoic, based on the existing sediments and sedimentary rocks.

ratios below 5. The decline in the ratio from the Devonian into the Carboniferous is probably related to the development of woody land plants which produce carbon compounds that are resistant to decomposition. The sharp rise in the ratio at the end of the Permian may be related to the development of fungi or other organisms capable of decomposing lignin. There is no obvious relation between the changes in the carbonate/organic carbon ratio and model reconstructions of atmospheric CO₂ levels (Berner et al. 1983; Berner 1991, 1994; Berner and Kothavala 2001; Wallmann 2001). Before the Late Mesozoic, most carbonate was probably deposited, at least initially, on low latitude continental shelves, epeiric seas, and oceanic platforms. The amount of carbonate that could be deposited would be limited by the available accommodation space. Accommodation space for carbonate sediments should be related to sea level, but the large sea-level rises of the Paleozoic and Mesozoic are not reflected in the carbonate/organic carbon ratio. Organic carbon is deposited mostly in shallow water fine-grained detrital sediments, although there may have been times when low oxygen contents of deeper ocean waters allowed more widespread deposition of C_{org} in the deep sea. Again, deposition of C_{org} might be expected to relate to accommodation space available for shallow water deposits, but more importantly it is probably related to the supply of fine-grained detritus which is controlled primarily by topographic relief. However, the carbonate/organic carbon ratio does not reflect the changes in overall sediment mass, shown in Figs 1 and 2. Another possibility is that the carbonate/organic carbon ratio reflects evolution of carbonate secreting organisms, but nothing certain is known about the quantitative aspects of this possibility. In summary, although the carbonate/organic carbon ratio varies by a factor of four during the Phanerozoic, there is no obvious long-term trend and the cause of the variations is not known.

Phanerozoic carbonates existing today and in the past

Carbonate rocks make up 13 to 25% of the total sediment representing units of the Phanerozoic compiled by Ronov and his colleagues. The Ronov data show a remarkable feature with respect to carbonate rocks on the continental blocks – they are most abundant in the Paleozoic, and their abundance declines particularly during the Cenozoic as can be seen in Fig. 4. This is in contrast to terrigenous sediments, which appear to become more abundant with time.

Budyko and Ronov (1979) proposed that the history of the atmosphere can be interpreted from the sedimentary record, with the O₂ content of the atmosphere being proportional to the amount of organic carbon buried, and the CO₂ content proportional to the amount of carbonate rock deposited. They also observed that the abundance of carbonates paralleled variations in the abundance of volcanic rocks through time. From the abundance of carbonates existing today they projected the atmospheric content of CO₂ at the time the rocks were deposited, concluding that CO₂ levels in the Cretaceous were many times the present concentra-

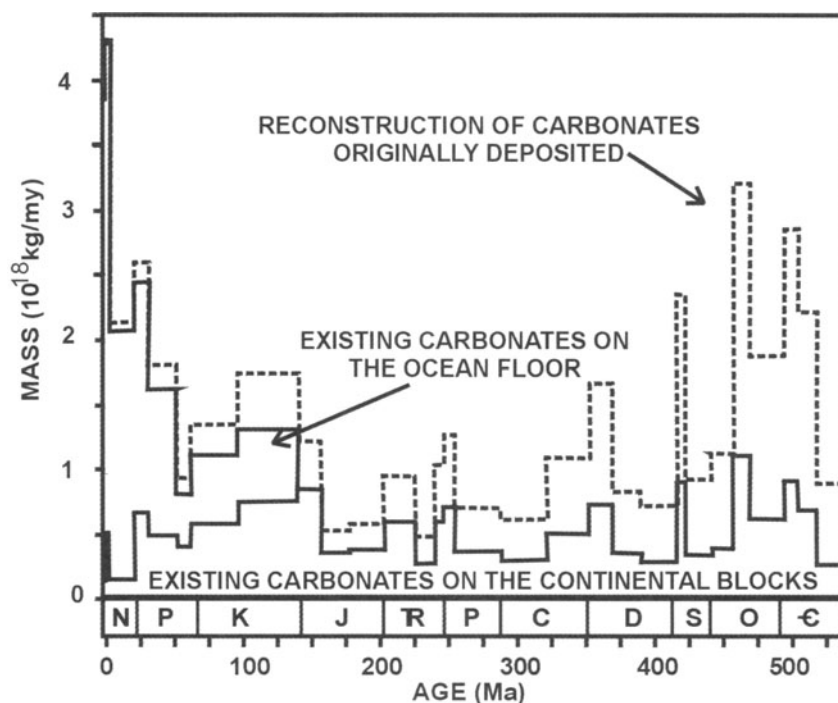


Fig. 4. Existing carbonates on the continental blocks and on the ocean floor (solid lines), and the reconstruction of carbonates originally deposited, assuming that the proportion of carbonate to total sediment in each time interval has not changed.

tion. They argued that this was the cause of the warm equable climate of the late Mesozoic. Hay (1985) noted that Budyko and Ronov (1979) had based their argument on the existing masses of carbonate rock on the continental blocks, and if the carbonates in the deep sea are included, it appears that the accumulation rate of carbonate has actually increased since the Cretaceous. It should be noted, however, that Budyko and Ronov's (1979) suggestion that atmospheric CO_2 levels are intimately related to volcanism has become widely accepted (e.g. Berner et al. 1983; Berner 1991, 1994; Wallmann 1999, 2001; Berner and Kothavala 2001). Budyko et al. (1987) followed this hypothesis in their discussion of the history of the atmosphere and climate.

The general Phanerozoic history of carbonate deposition has been discussed by Hay (1999). The effect of reconstructing the ancient sediment masses originally deposited is to amplify the amounts of carbonate rock that must have existed in the past, particularly in the Paleozoic for which only about 1/3 of the originally deposited sediments remains. This poses two important problems – where did the early Phanerozoic carbonates come from, and why have the masses of carbonate deposited on the continental blocks declined with time. At least the Cambrian and Ordovician carbonates must have come from erosion of pre-existing Precambrian

carbonates. The problem is that carbonates are relatively rare in the Precambrian. This peculiar situation may be related to the possible late Precambrian glacial rises (Snowball Earth, Hoffmann et al. 1998, see also http://www-eps.harvard.edu/people/faculty/hoffman/snowball_paper.html).

The causes of the decline in the amount of carbonate on the continental blocks

The most plausible explanation of the decline in carbonate deposition on the continental blocks through the Phanerozoic has been presented by Walker et al. (2002). From analysis of the Ronov and other data they found that although there are large variations associated with long-term trends of sea level change, the area of shelf carbonate deposition has decreased from about $36 \times 10^6 \text{ km}^2$ in the early Cambrian to about $0.6 \times 10^6 \text{ km}^2$ today. Noting that at present carbonate deposition on continental shelves occurs mostly in the tropics between 30°N and S , they analyzed the areas of shelf between these latitudes shown on the series of Phanerozoic paleogeographic maps of Scotese and Golonka (1992). They found that the area of tropical shelves has decreased from about $44 \times 10^6 \text{ km}^2$ in the early Cambrian to about $15 \times 10^6 \text{ km}^2$ today, again with major variations reflecting long-term sea level trends. They conclude that the reduction of tropical shelf accommodation space for carbonate deposition has forced a long-term shift of carbonate deposition from the shelves to the deep sea.

What was the nature of the shift of carbonate deposition from the shelves to the deep sea? In Paleozoic and early Mesozoic ophiolites pillow basalts are generally overlain by dark shales and cherts (Nicolas et al. 2001). This implies that during this time there were no pelagic organisms producing carbonate tests that survived dissolution to be deposited on the mid-ocean ridge system. The alternative is that carbonate was shed from shallow shelves and carbonate platforms via turbidity currents and mass wasting. It is known that shallow water carbonate production by algae today, can greatly exceed the accommodation space available for deposition on shelves and platforms. Excess material is swept off these regions during storms and is deposited in bordering deep sea aprons (e.g. Pilska et al. 1989; Glaser and Droxler 1991; Schlager et al. 1994). Presumably, most of this deep-sea carbonate material has been subducted. Interestingly, geochemical models (e.g. Berner et al. 1983; Berner 1991, 1994; Wallmann 1999, 2001; Berner and Kothavala 2001) assume that carbonate sediments on the deep-sea floor have been subducted during the entire Phanerozoic to provide for the CO_2 flux from volcanics.

When did the pelagic carbonate rain originate and what were its components? At present the pelagic carbonate rain consists of the aragonitic shells of pteropods, the calcitic tests of planktic foraminifera and calcitic coccoliths. The pteropod shells, being aragonitic, are usually dissolved in the water column above the aragonite compensation depth (about 300 m in the Pacific, 1 to 2 km in the Atlantic according to Ramsay 1974) or at the sediment surface and are only rarely incorporated into the sediment. The oldest pteropod remains are Late Cretaceous.

Small tubular or conical shells in Early Paleozoic sediments were originally thought to be related to pteropods, but are now considered to belong to other groups. They remain curiosities and did not form deep-sea oozes. The oldest planktic foraminifera are middle Jurassic, but they did not become common until the Cretaceous, becoming widespread throughout the ocean at the end of the Early Cretaceous. Although all of the planktic foraminifera produce tests made of calcite, they are differentially soluble (Berger 1967, 1968, 1970 and subsequent papers). Within a relatively thin zone, termed the “lysocline” by Berger (1968, 1970) the more resistant tests are dissolved. The calcareous nannoplankton appeared in the latest Triassic, but evidence from mountain belts, ophiolite sequences, and the few deep-sea sediment samples of Jurassic material recovered by the DSDP and ODP indicates the throughout most of the Jurassic they were restricted to shelf seas (Roth 1986). Starting in the latest Jurassic they spread into the open ocean. As shown in Fig. 5, the spread of calcareous plankton coincides with major changes in ocean salinity as a result of salt extractions in the Atlantic and Gulf of Mexico (Floegel et al. 2000; Hay et al. 2001). The evolutionary appearance of solution-resistant coccoliths in the Cretaceous, particularly *Watznaueria barnesae*, changed the shallow water-deep water carbonate balance in the ocean.

As with the planktic foraminifera, calcareous nannoplankton are differentially soluble (Schneidermann 1977) and a case can be made that the most soluble forms, holococcoliths, dissolve in the upper waters of the ocean and that there is a continuous spectrum of dissolution of species with depth. In spite of their small size, some coccoliths are more solution-resistant than foraminifera. Below the lysocline, where resistant planktic foraminifera are dissolved, and above the calcite compensation depth calcareous nannoplankton fossils form coccolith ooze. In summary, the large scale deposition of pelagic calcareous oozes on the deep-sea floor began in the Early Cretaceous. With the Late Cretaceous highstand of sea level, deposition of these pelagic calcareous oozes spread into the shelf and epicontinental seas leaving vast deposits of chalk.

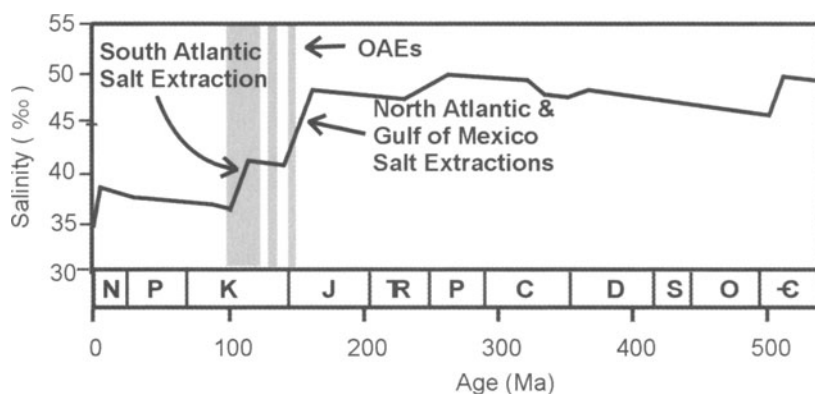


Fig. 5. Changes in mean ocean salinity during the Phanerozoic, based on sedimentary mass balance (after Floegel et al. 2000; Hay et al. 2001).

Another, less likely, possibility is that the uptake of Ca^{++} and HCO_3^- during alteration of ocean crust has changed with time. Wallmann (2001) has noted that this is a significant sink, amounting to as much half of the hydrothermal Ca^{++} flux. The long term behavior of this sink is not known.

The balance between carbonate deposition and dissolution

Today the surface ocean is supersaturated and the deep ocean undersaturated with respect to both major phases of CaCO_3 , aragonite and calcite. The German Meteor Expedition of 1925 in the South Atlantic collected samples that showed a distinctive depth-related boundary between carbonate ooze and red clay. This boundary was originally termed the "calcium carbonate compensation depth". Most of CaCO_3 precipitation is biologically mediated, and the CaCO_3 produced by each species is structurally different so that the solubilities differ. An extreme example is the aragonite spicules of gorgonians, soft corals that are abundant in tropical waters. Vaughan (1919) had noted that the rate of production of these spicules is very high, but they are not found in the sediment in spite of the fact that the waters are supersaturated with respect to mineral aragonite. As noted above, the tests of different species of planktic foraminifera and the coccoliths and other skeletal elements produced by coccolithophores have differential solubilities.

The positions of different dissolution levels in the ocean, aragonite compensation depth (ACD), planktic foraminiferal lysocline, calcite compensation depth (CCD), shown schematically in Fig. 6, depend on the temperature and amount of dissolved CO_2 in different water masses, pressure, and the overall chemistry of the ocean waters. Most of these factors have changed markedly with time, although they have yet to be documented in detail. Berger and Winterer (1974) suggested that the long-term fluctuations of the calcium carbonate compensation depth (CCCD) follow broad trends of sea-level change. Throughout the Phanerozoic the deeper parts of the ocean have probably been corrosive to carbonate, and it would be expected that at times when there were higher levels of atmospheric CO_2 , the compensation depths would be shallower. Van Andel et al. (1975, Fig. 6) showed that during specific time intervals the rate of calcium carbonate accumulation decreases more or less steadily with depth, implying increasing solution with depth. Further, they found that the rate of dissolution versus depth changes with time. Unpublished analysis of DSDP (Deep Sea Drilling Project) data by this author showed that this was characteristic of each ocean basin, but that there were differences between the ocean basins, and major differences between time intervals. The Oligocene was a time when the different compensation depths must have been very close to each other.

How much of the ocean flux of CaCO_3 is in the form of coccoliths and other skeletal particles secreted by coccolithophores? The only data that are useful in making an estimate are the "sand-silt-clay" ratios from calcium carbonate-rich

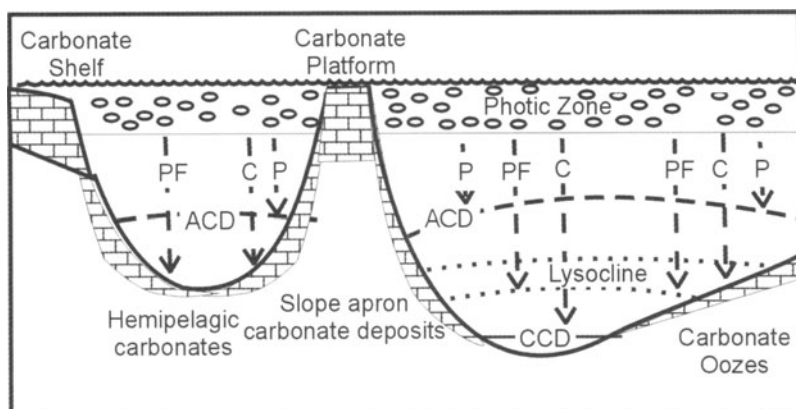


Fig. 6. Schematic diagram of carbonate deposition and dissolution in the sea. See text for discussion. P = Pteropod shells, PF = tests of planktic foraminifera, C = coccoliths and other skeletal objects produced by calcareous nannoplankton. ACD = Aragonite Compensation Depth, CCD = Calcite Compensation Depth. Compensation depths are depressed beneath regions of high carbonate productivity.

sediments recovered by the ocean drilling programs. In oceanic carbonate sediments, “sand” means tests of planktic foraminifera, “silt” means fragments of tests of planktic foraminifera and some large calcareous nannoplankton remains, and “clay” is essentially the calcareous nannoplankton component. In sediments well above the lysocline, the sand to clay ratio is about 1:1, below the lysocline, it approaches 0:1. The integrated values suggest a ratio of 1:2, implying that about 2/3 of the oceanic carbonate flux is in the form of calcareous nanofossils.

Carbonate deposition on different time-scales

As discussed above, over the long-term the supply of calcium and carbonate to the oceans from rivers and hydrothermal fluxes along the mid-ocean ridge must equal the output as carbonate sediment deposited on the sea floor and carbonate within the hydrothermal systems. In this context, long-term can be defined as equal to the residence time for Ca^{++} , which for these two sources is of the order of 400,000 years. On the shorter term input and output need not be equal.

Based on analysis of Deep Sea Drilling Project data, Hay and Southam (1977) estimated the average Late Mesozoic–Cenozoic flux of calcium carbonate into the deep sea to be about $0.8 \times 10^{12} \text{ kg yr}^{-1}$. More detailed analysis of the data by Hay et al. (1988) resulted in higher estimates for the Late Neogene and Quaternary of about $2.4 \times 10^{12} \text{ kg yr}^{-1}$, Early Neogene about $1.6 \times 10^{12} \text{ kg yr}^{-1}$, and Paleogene about $0.9 \times 10^{12} \text{ kg yr}^{-1}$; the Late Cretaceous average rate, based on spotty data, would be about $0.7 \times 10^{12} \text{ kg yr}^{-1}$. The present river flux of “ CaCO_3 ” (as Ca^{++} +

2HCO_3^-) to the ocean is about $1.2 \times 10^{12} \text{ kg yr}^{-1}$. The hydrothermal flux of Ca^{++} is now thought to be approximately equal to the riverine flux, so that the long-term inputs and outputs of CaCO_3 for the ocean during the Late Neogene and Quaternary are in balance.

Hay and Southam (1977) made detailed estimates of the average Neogene and Holocene carbonate fluxes onto reefs, the continental shelves, and platforms. Their estimate for the late Neogene was that the total reef, shelf and platform flux was between $0.07 \times 10^{12} \text{ kg yr}^{-1}$ and $0.26 \times 10^{12} \text{ kg yr}^{-1}$. This indicates that the late Neogene flux onto the shelves and platforms was not much greater than 10% and possibly as little as 3% of the total oceanic flux. They estimated that deep marginal seas (Red Sea, Black Sea, and the Mediterranean) received $0.1 \times 10^{12} \text{ kg yr}^{-1}$ and the continental slopes about $0.46 \times 10^{12} \text{ kg yr}^{-1}$. This implies that the deep sea received between 66 and 74% of the calcium carbonate reaching the ocean. Their estimate for the Holocene flux, greatly enhanced by the accommodation space that had formed during the glacial sea-level lowstand, was very different. They estimated the flux onto reefs, shelves, and platforms to be $0.74 \times 10^{12} \text{ kg yr}^{-1}$, into the tropical marginal seas to be $0.11 \times 10^{12} \text{ kg yr}^{-1}$, and onto the continental slopes to be about $0.46 \times 10^{12} \text{ kg yr}^{-1}$, for a total of $1.31 \times 10^{12} \text{ kg yr}^{-1}$. They noted that this would exceed the riverine flux, and assumed that it might draw down the global carbonate reservoir on the short term, but we now realize that this integrated Holocene flux is still less than the total riverine and hydrothermal flux. However, most of the Holocene flux into shallow water occurred in a few thousand years, shortly after the sea level rise, so it is possible that on very short time-scales the output might exceed the input. Unfortunately there is no detailed analysis of Holocene ocean sedimentation rates that would permit a global estimate of the open ocean flux and its possible changes in response to the changes in the shallow water flux.

More recently Milliman (1993) and Milliman and Droxler (1996) have made new compilations of modern calcium carbonate production and accumulation rates. They estimate the modern neritic accumulation flux onto coral reefs, onto banks and into bays, onto carbonate and non-carbonate shelves to total about $1.3 \times 10^{12} \text{ kg yr}^{-1}$. They estimated the accumulation flux onto continental slopes, imported from shallower areas, to be $0.4 \times 10^{12} \text{ kg yr}^{-1}$. These estimates are thought to be accurate within a factor of 1, and do not greatly differ from those of Hay and Southam (1977). They estimated the modern (Holocene) deep-sea accumulation flux to be about $1.1 \times 10^{12} \text{ kg yr}^{-1}$, significantly less than the value for the long term flux given by Hay and Southam (1977). Iglesias-Rodriguez et al. (2002) have summarized the present state of knowledge of the ocean's calcium carbonate budget, but the calculations are based in part on chemical properties of the ocean water (alkalinity) which may already be affected by uptake of fossil fuel CO_2 .

Fig. 7 shows four possible schematic scenarios for the shallow and deep water carbonate fluxes. Fig. 7A represents the condition during glacial low sea level stands, when the shoreline has retreated below the edge of the shelf break in the tropics. The entire carbonate flux goes into the deep sea. Figs 7B and 7C represent two alternative views of the condition during the interglacial high sea-level stands.

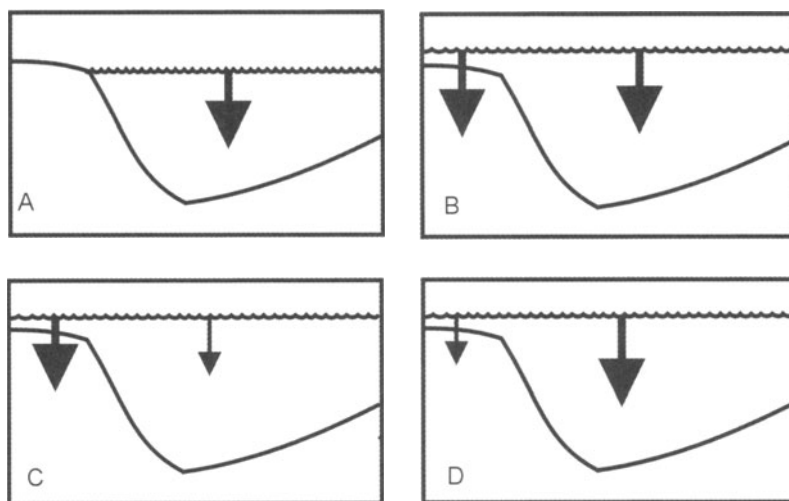


Fig. 7. Alternative scenarios for CaCO_3 deposition in the sea with changing sea levels. For discussion see text.

In Fig. 7B the large flux into the deep sea continues undiminished, but there is an additional, short-lived, flux into shallow water areas to fill the available accommodation space. In Fig. 7C the oceanic flux is shown to be reduced to accommodate the great flux onto the flooded shelves and platforms. Fig. 7D shows the relation of the shelf-platform and oceanic fluxes after a long period of high sea-level stand, i.e. during the Neogene before the large sea-level falls associated with the northern hemisphere glaciation.

The effect of subduction of carbonate sediment

Carbonate deposited today on the ocean floor will ultimately be subducted. What will be its fate? Does it become incorporated into accretionary wedges? Apparently not, because there are very few records of calcareous oozes obducted into accretionary wedges. Is it decomposed, do the Ca and the CO_2 come back through volcanoes? In addition to the CaCO_3 in pelagic deposits, CaCO_3 is precipitated within the oceanic crust (Alt and Teagle 1999). The Ca in andesitic rocks is depleted relative to oceanic basalts, so the Ca flux is apparently into the mantle (Southam and Hay 1981). The CO_2 might be returned in part or in its entirety through volcanoes. The GEOCARB models (Berner 1991, 1994; Berner and Kothavala 2001) assume that CO_2 from volcanoes along subduction zones is a major source of CO_2 for the atmosphere, and that the rate of output is a function of both the seafloor spreading rate and the pelagic CaCO_3 accumulation rate which they assume to have increased 100 million years ago. Wallmann (2001) has noted

that today the rate of accumulation of pelagic carbonate is much greater than the rate of its subduction because most of it is deposited in the Atlantic which is bordered by passive margins. He concluded that only a part of the carbonate entering the subduction zone is returned to the atmosphere through volcanic activity. The remaining carbonate may be subducted into the mantle where it could form scapolites, minerals having the formula $\text{CaCO}_3 + \text{feldspar}$, which are stable in the mantle (Southam and Hay 1981). If scapolite formation is the ultimate result of much of the subduction of CaCO_3 the spread of calcareous plankton into the ocean may be regarded as a sort of "doomsday machine" removing carbon from the surface of the Earth and storing it in the mantle.

Summary and Conclusions

Most of the sediments being deposited at any given time are derived from the erosion of older sediments. Accordingly, the existing masses of most sediments decrease with age. However, carbonates on the continental blocks show the opposite trend. Very large amounts of carbonate must have been recycled from the Precambrian into the Early Paleozoic. The general loss of carbonate from the continental blocks has been attributed to the decline in area and accommodation space on tropical shelves during the Phanerozoic as a result of continental drift.

There is no record of deposition of significant amounts of pelagic carbonate in the deep sea prior to the Cretaceous. During the Paleozoic and earlier Mesozoic the delivery of carbonate to the deep sea must have been through turbidity currents carrying fine-grained skeletal and inorganically precipitated particles from shallow water banks and shelves into the depths. This phenomenon occurs today, and many oceanic banks and tropical shelves are bordered by aprons of aragonitic carbonate derived from shallow waters.

The large-scale pelagic rain of carbonate into the deep sea, both in the form of tests of planktic foraminifera and skeletal elements of calcareous nannoplankton, began in the Early Cretaceous and has continued ever since. The surface ocean is supersaturated with respect to both aragonite and calcite, and the deep sea is undersaturated, this has probably been the case throughout the Phanerozoic. Biogenically mediated carbonates have slightly different solubilities, so that the tests of different species of planktic foraminifera and the calcareous nannofossils tend to be dissolved in sequence, with the most resistant forms reaching greatest depths. Dissolution of pelagic carbonate in the ocean has changed with time, and the difference in depth between the major reference horizons, the aragonite compensation depth, lysocline, and calcite compensation depth has been at times much smaller than it is today.

Carbonate on the ocean floor will ultimately be subducted, but at present more carbonate is accumulating than is being subducted. This is because the major site of accumulation is in the Atlantic ocean, which is bordered by passive margins. Subducted carbonate is in part decomposed with the CO_2 returning to the Earth's surface through volcanoes. The remainder may be subducted into the mantle, per-

haps as scapolites, minerals having the composition CaCO_3 + feldspar. The pelagic carbonate rain produced by planktic foraminifera and calcareous nannoplankton is a geologically young feature of the sedimentary cycling system and is gradually differentiating the global sedimentary reservoir.

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Coccolithophorid-based geochemical paleoproxies

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Summary

Coccolithophores are the only marine organisms that provide indicators of past climatic and oceanographic conditions from both the organic (molecular fossils or biomarkers) and inorganic (calcium carbonate) remains in sediments. The under-saturation ratio of alkenone biomarkers (U_{37}^K) provides information about past sea surface temperatures and is gaining widespread use as a paleotemperature proxy, particularly in the Quaternary sediment record. The carbon isotopic fractionation in alkenone biomarkers ($\epsilon_{\text{alkenone}}$) should allow reconstruction of past dissolved and atmospheric CO_2 concentrations if independent proxies are able to consistently constrain the nutrient or growth rate influence on isotopic fractionation. The Sr/Ca ratio of coccolith carbonate is the most developed proxy from the elemental chemistry of coccoliths. Recent culture and field studies suggest that the Sr/Ca ratio has potential as an indicator of nutrient-stimulated coccolithophorid growth rates. In contrast, while the Mg/Ca ratio of coccoliths is probably controlled by temperature, formidable challenges in removing noncarbonate sources of Mg from coccolith fractions will probably preclude use of coccolith Mg/Ca for paleothermometry. Similar challenges in cleaning will probably also preclude use of coccolith Cd, Ba, V, or U. Stable isotopic measurements in coccolith-dominated bulk carbonate have been widely used to infer temperature changes and changes in the carbon cycle in the Mesozoic and Early Cenozoic, despite an array of nonequilibrium or “vital effects” in different species. In addition to paleoceanographic applications, continued study of the stable isotopic fractionation of coccoliths in culture may also elucidate mechanisms of carbon acquisition in different coccolithophorid species. As is the case for all paleoceanographic proxies, continued calibration studies are required to further improve our understanding of coccolithophorid-based proxy systems and increase confidence in their application.

Introduction

Both the organic and inorganic remains of coccolithophores provide key geochemical records for study of past oceanographic, environmental, and biological conditions. Coccolithophores are useful for paleoceanographic reconstructions because they are widespread throughout the ocean and both organic and inorganic remains enjoy long term preservation in the marine sediment record. The inorganic record is especially durable and extends back to the evolution of coccolithophores in the Early Mesozoic.

Until recently, the chemistry of the organic biomarkers from coccolithophores has been much more widely used in paleoceanographic studies than the chemistry of the inorganic (coccolith) sediment record of coccolithophores. The alkenones, organic biomarkers produced by a small group of coccolithophores, can be readily isolated from sediments by chromatographic extractions. For over a decade alkenones have served as the basis for two important paleoproxies, the undersaturation ratio of alkenones (U_{37}^K) which depends on temperature (Brassell et al. 1986), and the carbon isotopic fractionation, $\epsilon_{\text{alkenone}}$, used to reconstruct past atmospheric CO_2 concentrations (Jasper and Hayes 1990). In contrast, although coccoliths are a major and often dominant component of marine carbonate sediments, and as such have been used implicitly in studies relying on bulk geochemical records, the deliberate selection of coccoliths for elemental and stable isotopic analysis is a relatively recent paleoceanographic strategy. Most geochemical studies of marine carbonate have relied on foraminifera, since individual coccoliths are too small to be picked individually for geochemical analysis. However, recent work suggests that the elemental chemistry of coccoliths can provide unique proxies for past variations in coccolith productivity (e.g. Stoll and Schrag 2000). Recent culture studies also suggest the potential for new insights from coccolith stable isotope chemistry (e.g. Ziveri et al. 2003). Table 1 summarizes the major coccolithophore-based chemical proxies reviewed in this chapter.

As Table 1 indicates, most paleoproxies are not controlled by a single variable of paleoceanographic interest. Other secondary factors typically also influence the proxy, and the influence of these secondary factors must be constrained by independent means. For this reasons, studies combining multiple proxies are likely to be the most powerful. Among marine organisms, coccolithophores are the only ones with the potential to combine multi-proxy geochemical records from both organic and inorganic remains of the same organisms.

This chapter provides a brief overview of the organic proxies derived from coccolithophores, since these were recently the subject of a comprehensive review in a special theme of *Geochemistry, Geophysics, Geosystems* (Eglinton et al. 2001). We provide a more extensive review of proxies derived from the chemistry of the carbonate produced by coccolithophores, which have not been recently synthesized elsewhere.

Table 1. Summary of indicators derived from the chemistry of coccoliths and coccolithophorid biomarkers (alkenones).

	Indicator	Inferred primary control	Secondary influences	Limitations
Organic	U_{37}^K (alkenone undersaturation)	Temperature	Physiology or growth rate?	
	$\epsilon^{13}C$ alkenone	pCO_2	Cell growth rate and cell size	Requires estimation of $\delta^{13}C$ of dissolved inorganic carbon
	Sr/Ca	Productivity/coccolithophorid growth rate	Temperature and seawater Sr/Ca	Long term records ($>10^6$ yr) may require correction for changing seawater Sr/Ca
Inorganic (coccolith)	Mg/Ca	Temperature	Species-specific effects	Requires thorough cleaning of Mg from noncarbonate fractions
	$\delta^{18}O$	Temperature and $\delta^{18}O$ of seawater	Vital effects	May require separation of monospecific samples, assumption of constant vital effects, and inference of vital effects for extinct species
	$\delta^{13}C$	$\delta^{13}C$ of dissolved inorganic carbon	Vital effects	

Indicators from the chemistry of alkenone biomarkers

Certain species of coccolithophores produce unique organic compounds or biomarkers that have important applications in paleoceanography. Calcifying coccolithophores *Emiliania huxleyi* and the closely related *Gephyrocapsa oceanica* produce a suite of long-chain (C_{37} – C_{39}) unsaturated ethyl and methyl ketones known as alkenones. Although non-calcifying haptophytes *Cryptosilla* and *Isochrysis* also produce alkenones, these species are restricted to coastal waters and are not believed to be a significant source of alkenones in open ocean sediments (Marlowe et al. 1990). Alkenones are resistant to degradation and preserve well in sediments. The oldest alkenones extracted to date were recovered from Cretaceous black shales (Farrimond et al. 1986) and were probably produced by ancestors of the *Gephyrocapsaceae* family responsible for modern alkenone production (Marlowe et al. 1990).

Alkenone undersaturation as an indicator of sea surface temperature

Alkenones are most widely used in reconstructions of past sea surface temperature (SST). In the mid-1980's, researchers discovered that in the C_{37} methyl ketones,

the ratio of diunsaturated ($C_{37:2}$) and triunsaturated ($C_{37:3}$) methyl ketones reflects the growth temperature of the alkenone producers (Marlowe et al. 1984; Brassell et al. 1986). The alkenone undersaturation ratio is typically expressed as:

$$U_{37}^K = [C_{37:2}] / [C_{37:2} + C_{37:3}]$$

Several studies of laboratory cultures and sediment core tops have provided a series of calibrations between sea surface temperature and U_{37}^K (for a thorough review see Eglinton et al. 2001). These studies also revealed that a number of factors may influence the reliability of the U_{37}^K undersaturation ratio for SST reconstructions. Alkenone-production may occur tens of meters below the sea surface, biasing SST estimates to cooler values, and alkenone production may be highly seasonal in some areas. Some culture studies have demonstrated physiological effects on alkenone undersaturation based on the stage of growth or nutrients (Epstein et al. 1998) and on the strain cultured (Conte et al. 1998). Nonetheless, a compilation of global core top data by Muller et al. (1998) established a universal calibration between the mean annual sea surface temperature and alkenone undersaturation:

$$U_{37}^K = 0.033 \text{ SST} + 0.044$$

The consistency of the global calibration suggests that different calibrations in different strains or environmental conditions do not significantly detract from the overall reliability of the U_{37}^K temperature indicator. Consequently, alkenone undersaturation ratios are now widely used to reconstruct Quaternary sea surface temperatures (e.g. Bard 2001). The most reliable applications have been in open ocean areas with low to moderate sedimentation rates. Applications in high accumulation rate sediment drifts have been more complex since alkenones adhere to fine sediment particles and may have been transported from distant sites by bottom currents (Ohkouchi et al. 2002). Confidence in alkenone temperature estimates will improve as further studies identify the factors controlling alkenone biosynthesis and suggest settings where age offsets and non-temperature effects are likely to be least significant.

Carbon isotopic composition of alkenones for CO₂ paleobarometry

The difference in isotopic composition between alkenones and the dissolved inorganic carbon (DIC) of the seawater in which they are growing may permit calculation of the concentration of CO₂ dissolved in seawater, which is related to atmospheric pCO₂. However, alkenone CO₂ paleobarometry is more complex than alkenone undersaturation SST reconstruction. Additional proxies are required to estimate the isotopic composition of the dissolved inorganic carbon and constrain other factors influencing isotope fractionation. In addition, multiple calibration experiments have yielded contrasting results.

Theoretical models describe how the isotopic difference, or fractionation, between dissolved CO₂ and algal biomass (ϵ_p) responds to concentrations of CO₂. Carbon isotopes ¹³C and ¹²C are very strongly fractionated by the enzyme rubisco (ribulose-1,5-bisphosphate carboxylase) which catalyzes the photosynthetic fixa-

tion of CO_2 . Mass balance dictates that the net isotopic fractionation between the phytoplankton and CO_2 must reflect the efficiency of this carbon fixation, i.e. the proportion of carbon taken into the cell that is fixed via this enzyme relative to that which diffuses back into the surrounding water. This efficiency is believed to depend on the cell growth rate, cell permeability and geometry, and the concentration of CO_2 in the external seawater (Farquhar et al. 1982). Some additional isotopic fractionation may also occur during the uptake of inorganic carbon into the cell and its diffusion back into the surrounding water (Farquhar et al. 1982). As these theoretical relationships suggest, past concentrations of dissolved CO_2 can be inferred from isotopic fractionation in algal biomass only when these other factors can be constrained. While ϵ_p can be measured in bulk organic carbon from sediments, measurement of alkenone carbon isotopic fractionation ($\epsilon_{\text{alkenone}}$) constrains more of these variables since alkenones are restricted to a specific marine algal group with specific cell sizes and biochemical pathways.

Field and culture studies confirm theoretical predictions that the carbon isotope fractionation of alkenones reflect the concentration of dissolved CO_2 in seawater, cell growth rates, and nutrient levels (Popp et al. 1998; Bidigare et al. 1997). However, the absolute fractionation, and slope of the relationship between CO_2 and isotopic fractionation (known as “b”), have varied greatly among different culture experiments using different factors to regulate growth rates (see detailed review in Laws et al. 2001). These studies suggest that reliable alkenone CO_2 paleobarometry will require not only additional proxy records to constrain variations in growth rates and nutrient levels, but also an improved understanding of how these and other factors influence $\epsilon_{\text{alkenone}}$. Fortunately, field studies suggest a consistent correlation between the slope (“b”) of the CO_2 - $\epsilon_{\text{alkenone}}$ relationship and phosphate concentrations, which would enable calculation of CO_2 if a proxy for past PO_4 concentrations were available. One early study of the alkenone CO_2 paleobarometer appears to have overcome some of these limitations by working in an oligotrophic environment where past changes in nutrients and growth rates may have been negligible. For example, alkenone carbon isotopic compositions from an oligotrophic Gulf of Mexico core correlate with known Quaternary variations in atmospheric CO_2 for the measured 70,000 year long record (Jasper and Hayes 1990). While there are still many uncertainties to overcome in the alkenone paleobarometer proxy, it will probably remain an important approach because there are few alternative proxies for atmospheric CO_2 in the pre-Quaternary record.

Indicators from the elemental chemistry of coccolith carbonate

The elemental chemistry of marine biogenic carbonates has been widely used to reconstruct both past seawater chemistry and past seawater temperatures. In open ocean sediments, most studies focus on the chemistry of benthic and planktic foraminifera, whose larger sizes (typically 100 to 500 μm) permit the selection of individual foraminifera of particular species for geochemical analysis. The

chemistry of marine carbonates is controlled by 1) the concentration of the element in the seawater from which the carbonate is produced, along with 2) the partitioning of the element between seawater and carbonate which depends on thermodynamic, kinetic, and biological effects. Both types of effects can provide information of paleoceanographic interest. In the case of foraminifera, variations in many elemental ratios (e.g. Ba/Ca, Cd/Ca, U/Ca, V/Ca) are attributed to changing biogeochemical cycles which influence the abundance or distribution of these elements in the ocean (Boyle 1988; Lea and Boyle 1993; Russell et al. 1994; Hastings et al. 1996), and partitioning effects are typically assumed to be constant (some exceptions noted by Rickaby and Elderfield 1999; Stoll et al. 1999). In contrast, temperature effects may be the dominant control on Mg/Ca ratios in foraminifera, and foraminiferal Mg/Ca is emerging as a widely applied paleothermometer (e.g. Nuernberg et al. 1996). The Mg/Ca proxy is effective since Mg/Ca ratios of seawater are very homogeneous and change only slightly on time-scales less than a million years.

Proxies developed from the elemental chemistry of coccoliths are likely to differ from those of foraminifera in several ways. Unlike foraminifera, coccolithophores produce calcite intracellularly (see Billard and Inouye this volume). Consequently, coccolith calcite may have unique biological or "vital" effects on element partitioning which could provide different paleoceanographic information than the chemistry of extracellularly precipitated carbonate of foraminifera. Single coccoliths are too small to be separated individually from sediments so it is not possible to "pick" monospecific samples of coccoliths. Microfiltering or settling techniques have successfully separated near-monospecific fractions of coccoliths from some sediments (e.g. Minoletti et al. 2001; Stoll and Ziveri 2002) for geochemical analysis. However, it is not possible to separate coccoliths from similarly sized non-coccolith particles present in sediments. This is a potential problem if a significant component of an element is present in the noncarbonate fraction of the sediment (as might be expected for Cd, V, Ba, and possibly Mg). To date, only Sr/Ca and Mg/Ca ratios have been studied extensively in coccoliths.

Sr/Ca ratios in coccolith calcite

Strontium has a similar ionic charge and similar (though slightly larger) ionic radius to calcium and can substitute for calcium in carbonate lattice. The most extensive substitution and complete solid solution series is attained in the more open aragonite crystal structure but limited substitution is possible in calcite. Several studies in abiogenic calcites noted a strong direct link between Sr/Ca ratios and crystal growth rates in abiogenic calcite (Lorens 1981; Tesoriero and Pankow 1996). Consequently, Stoll and Schrag (2000) hypothesized that in coccolith calcite, the Sr/Ca ratio might record the rate of coccolith production, highly correlated with cell growth rates, and might provide a useful paleoceanographic tool for reconstructing past coccolithophorid productivity.

Sediment core top and culture studies of coccolith Sr/Ca variation

The first study of coccolith Sr/Ca used core top sediment samples from across a persistent productivity gradient in the central Equatorial Pacific (Stoll and Schrag 2000). This study analyzed Sr/Ca ratios in a fine ($<12\ \mu\text{m}$) polyspecific coccolith fraction and found that the Sr/Ca ratio varied across the upwelling transect, and that high Sr/Ca ratios coincided with high surface productivity and high nutrient concentrations at the axis of maximum upwelling intensity on the equator. Because the ratio of Sr/Ca in seawater is homogeneous throughout the ocean (de Villiers 1999), variations in coccolith Sr/Ca ratios in sediments must reflect changes in incorporation of Sr in coccolith carbonate. Across this transect, Sr/Ca ratios of the coccolith fraction were much more variable (15% variation) than those of foraminifera (2% variation). Absolute Sr/Ca ratios of the coccolith fraction (2.0 to 2.4 mmol/mol) were also nearly twice as high as those of the foraminiferal ($>63\ \mu\text{m}$) fraction or of foraminifera *Globorotalia tumida* (1.2 and 1.35 mmol/mol, respectively). Bulk carbonate Sr/Ca had intermediate compositions and a simple two component mixing equation suggested that coccoliths contributed a greater fraction of bulk carbonate at the equator. The greater variability of coccolith Sr/Ca compared to foraminiferal Sr/Ca in the same core is also characteristic of downcore records from the Quaternary (Stoll and Schrag 2000) and Paleocene (Stoll and Bains 2003).

Subsequent studies of core top sediments suggest that different coccolithophorid species show different responses to gradients in nutrients across upwelling zones. Differential settling techniques were used to separate multiple fractions of near-monospecific coccolith samples from sediments across productivity gradients in the Equatorial Pacific and Somali Coastal Upwelling Zone (Stoll et al. 2002b). In these transects, the amplitude of Sr/Ca variations is much larger in large coccolithophores like *C. leptoporus* (23–55%) than in smaller coccoliths of *G. oceanica* or *F. profunda* (6–15% variation in Sr/Ca) (Fig. 1; Stoll et al. 2002b). These different responses likely reflect the different ecology of the species; *F. profunda* lives in deeper waters whose nutrient levels may be higher and more constant across the upwelling transect than the nutrient levels in surface waters in which *C. leptoporus* and *G. oceanica* live. In both of these field regions studied to date, nutrient availability, rather than light availability, most likely limits coccolithophorid growth.

Further study of the Sr/Ca proxy in sediment core tops is desirable, since the environment is closest to that in which we wish to apply the paleoproxy.

However, core-top calibration in other areas been limited by the scarcity of measurements of primary productivity of specific algal groups like coccolithophores in many ocean settings. Consequently, Sr/Ca calibration studies have been confined to upwelling regions where there are strong and easily identifiable gradients in productivity.

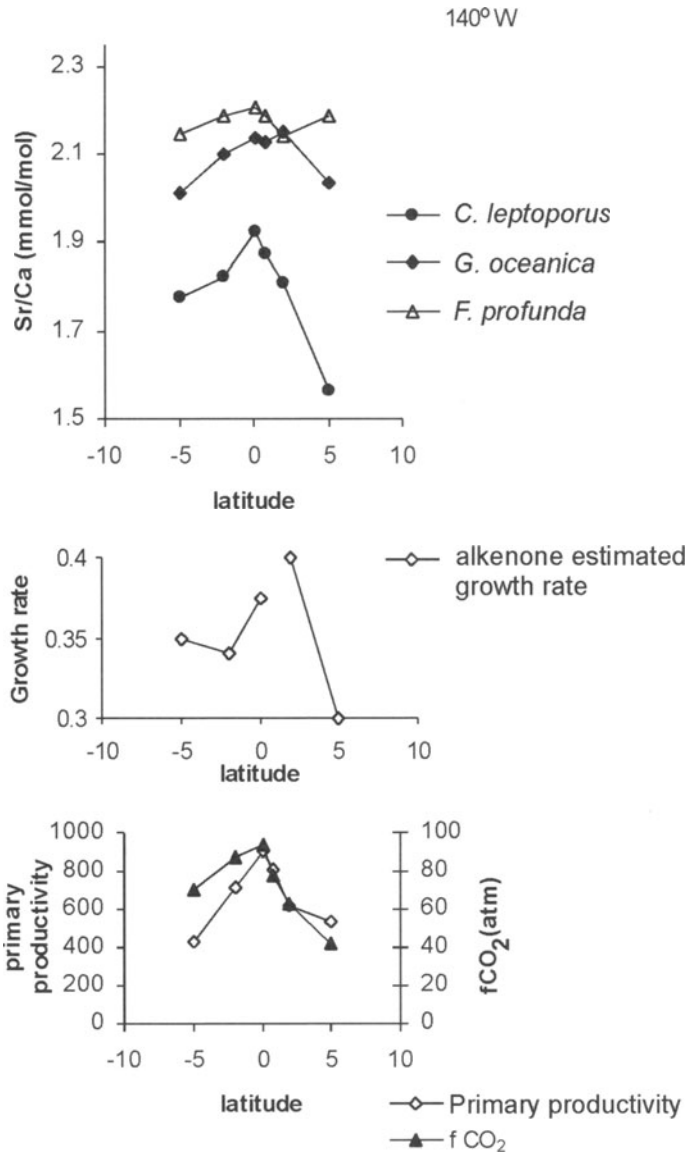


Fig. 1. Sr/Ca in monospecific samples separated from surface sediments in the equatorial Pacific upwelling region (upper panel) compared with indicators of productivity in modern surface waters (lower panels). Alkenone-estimated growth rates are cell division rates for alkenone producing haptophytes including coccolithophores *E. huxleyi* and *G. oceanica*; data from Bidigare et al. (1997). Primary productivity in $\text{mg C m}^{-2} \text{ day}^{-1}$ from Chavez et al. (1990), Barber et al. (1991) and Chavez et al. (1998). Fugacity of CO₂ (fCO₂) data in microatmospheres, from Takahashi et al. (1997).

Attempts to investigate the Sr/Ca ratio of coccoliths extracted from living populations in surface waters have not yet been successful. Surface water particulates collected off the coast of Portugal contained particles of Sr-rich celestite (SrSO_4) from acantharia that could not be separated from the coccolith particles for chemical analysis (Stoll et al. unpub. data). Fortunately, celestite is highly soluble and is typically absent from marine sediments.

Culture studies of coccolith Sr/Ca

Culture studies have generally verified increasing Sr/Ca ratios with increasing growth rates, although the slope of the response has been variable in different culture experiments. Several continuous and batch culture experiments under varied light levels (at constant temperature and moderate nutrient levels) produced a large range of steady-state coccolithophore cell division and calcification rates. In *E. huxleyi*, large increases in cell division and calcification rates led to only modest (10%) increases in coccolith Sr/Ca (Fig. 2a and Table 2; Stoll et al. 2000a, 2000b, 2002c). Comparable Sr/Ca variations in the closely related species *G. oceanica* were attained over a much smaller range in inferred growth rates in the Equatorial Pacific sediments (Table 2). Nutrient limited batch culture of *E. huxleyi* produced a much steeper response of Sr/Ca to changing growth and calcification rates (Fig. 2b). Sr/Ca ratios decreased by a factor of six as cell division rates and nutrient contents decreased while the culture passed from early through late exponential phase (Rickaby et al. 2002). This response was identical for replicates grown at lower salinity or higher pCO_2 . Different responses depending on the factor limiting growth is generally consistent with previous studies which have shown that the biochemical composition of phytoplankton at a given growth rate is very dependent on the factor limiting growth (Shuter 1979).

Culture studies also show that temperature can exert an important secondary influence on coccolith Sr/Ca ratios. Culture experiments at different temperatures (but constant light levels) showed a significant increase in Sr/Ca ratios with temperature, of about 1% per degree C (Stoll et al. 2002a, 2002b; Fig. 2c). This increase is in addition to the increased Sr/Ca ratio due to higher growth rates at higher temperatures. The slope of this relationship is similar to that observed in abiogenic calcites (Malone and Baker 1999) and in cultures of planktic foraminifera (Lea et al. 1999) and may be a ubiquitous thermodynamic effect in all calcites that must be considered in paleoceanographic applications of coccolith Sr/Ca. The temperature effect on Sr/Ca appears to have been overwhelmed by the productivity effect in the sediment core top studies in the Equatorial Pacific setting, since Sr/Ca ratios at the equator were higher despite sea surface temperatures that were nearly 4°C lower than at the sites off the axis of upwelling.

Different coccolithophore species grown under identical culture conditions produce coccoliths with Sr/Ca ratios varying about 20% (Fig. 2d; Stoll et al. 2002b, 2002c). Early culture studies comparing Sr/Ca among different species (Stoll et al. 2002c) showed a positive correlation between Sr/Ca and interspecific variations in calcite production rate and organic C fixation rate. However,

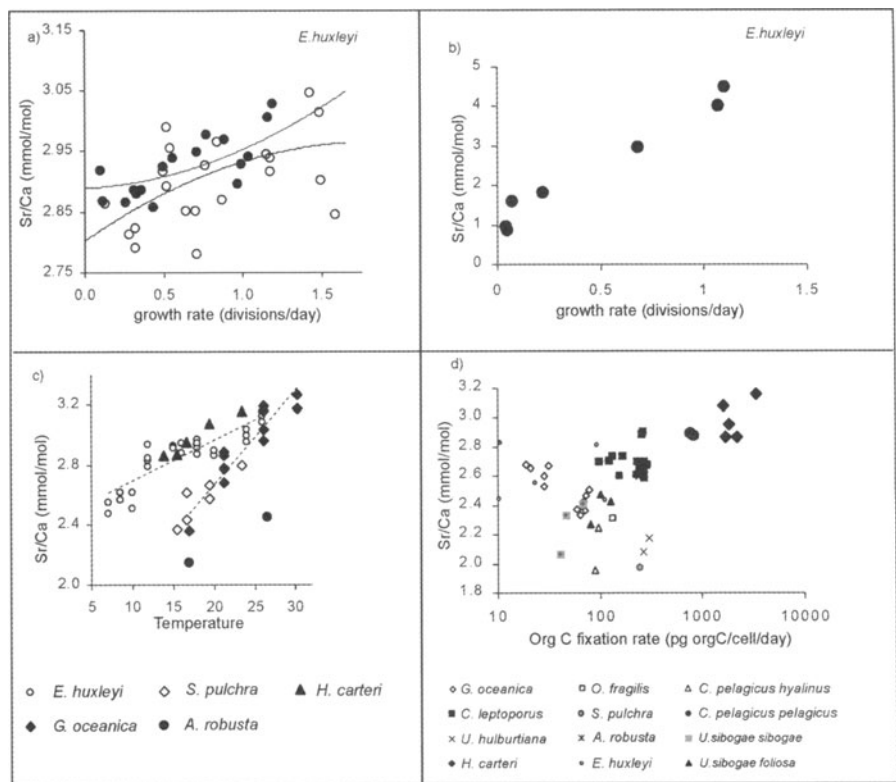


Fig. 2. Summary of coccolith Sr/Ca results from culture studies. **a)** Sr/Ca of coccoliths of *Emiliania huxleyi* from 18°C continuous culture experiments at low (f/50; open circle) and high (f/2; filled circle) nutrient enrichments vs. growth rate ($r^2 = 0.22$ (all data), $r^2 = 0.58$ (f/2), $r^2 = 0.23$ (f/50)). Lines indicate 95% confidence interval on the regressions; all statistics are for geometric mean regression analysis (reduced major axis). Data from Stoll et al. 2002a. **b)** Sr/Ca of coccoliths of *Emiliania huxleyi* from 15°C batch culture experiments. Data from Rickaby et al. (2002); Sr/Ca ratios are calculated for precipitation from seawater using reported partitioning coefficients. **c)** Sr/Ca vs. temperature in a range of batch and continuous culture experiments (Stoll et al. 2002b). Linear best fits are shown for *E. huxleyi* ($y = 0.0272x + 2.4238$; $r^2 = 0.78$) and *G. oceanica* ($y = 0.0636x + 1.3944$; $r^2 = 0.88$). **d)** Sr/Ca ratios in all batch cultures vs. organic carbon fixation rate estimated from cell biovolume and growth rates (Stoll et al. 2002b). Sr/Ca ratios in a), b), and c) have been corrected for small variations in media Sr/Ca.

additional species cultured in later studies do not follow these trends as closely, indicating that a simple calcification rate or organic C fixation rate control cannot be invoked to explain both interspecific and intraspecific variations in coccolith Sr/Ca. Although the overall correlation is not strong, the largest species with highest organic C fixation rates (*C. leptoporus*, *C. pelagicus*, and *H. carteri*) show the highest Sr/Ca ratios in culture.

Table 2. Summary of selected relationships between Sr/Ca and growth rate. For all experiments, the range in Sr/Ca is calculated to reflect precipitation from seawater. For the field study, growth rates were estimated from carbon isotopic fractionation in alkenones by Bidi-gare et al. (1997).

Species	Growth Regulation	Range in growth rate (day ⁻¹)	Range in Sr/Ca (mmol/mol)	Reference
<i>E. huxleyi</i>	Light (culture)	0.1 to 1.6	2.8 to 3.05 (10%)	Stoll et al. 2002a
<i>E. huxleyi</i>	Nutrients (culture)	0.03 to 1.1	0.85 to 5 (600%)	Rickaby et al. 2002
<i>G. oceanica</i>	Nutrients? (Eq. Pac.)	0.3 to 0.4	2.01 to 2.15 (6%)	Stoll et al. 2002b

It is difficult to compare directly the Sr/Ca response of cultured coccoliths with those of foraminifera because there are no paired measurements of Sr/Ca ratios and calcification or growth rates for cultured foraminifera. Small increases in Sr/Ca with increasing pH are hypothesized to reflect more rapid calcification and increased incorporation of Sr at higher pH and higher carbonate ion concentrations (Lea et al. 1999).

Why does coccolith Sr/Ca vary?

Variations in Sr/Ca ratios of coccoliths (Stoll and Schrag 2000), foraminifera (Lea et al. 1999) and biogenic carbonates in general (Carpenter and Lohmann 1992) have been attributed to the same kinetic mechanisms that cause Sr/Ca to vary with calcification rate in abiogenic calcites (e.g. Lorens 1981; Tesoriero and Pankow 1996). Such kinetic effects are believed to arise because surfaces of growing crystals have higher equilibrium concentrations of Sr than the lattice, and at more rapid crystal growth rates a higher proportion of this Sr is trapped in the interior of the crystal (e.g. Watson and Liang 1995). Data on Sr partitioning and calcite production rate in coccolith cultures permit the first comparisons between model-predicted kinetic behavior and Sr partitioning in coccoliths.

The surface enrichment model developed by Watson and Liang (1995) and Watson (1996) described kinetic phenomena numerically in terms of a competition between the rate of burial of the enriched surface layer by crystal growth, and the rate of return to equilibrium lattice concentrations by solid-state diffusion of the minor element out of the lattice. At the low temperatures at which coccoliths are produced, dissolution and reprecipitation in near-surface regions of the crystal, rather than solid state diffusion, likely accomplishes this return to equilibrium lattice concentrations but the parameterization of the process in the model would be identical. If calcification in coccolithophores is a relatively open system, with the supply of ions significantly exceeding the amount of calcite precipitated from them, then these kinetic effects are likely to be important in coccoliths.

The principle challenge in applying this model to coccoliths lies in the estimation of linear crystal growth rates from coccolith calcite production rates. Stoll et

al. (2002a) estimated minimum linear crystal growth rates, assuming that crystal growth occurred through the entire coccolithogenesis cycle, although crystal growth may represent only part of this process and therefore may be faster.

Given these assumptions, the growth rates of coccoliths plot along the relatively flat part of the curve of model-predicted Sr/Ca variations with crystal growth rate (Fig. 3a). *E. huxleyi* cultures grown at constant temperature but variable light showed a comparably gradual slope of increasing Sr/Ca with increasing growth rate and could potentially be explained by this surface enrichment model. However, the high Sr/Ca ratios of coccoliths compared to abiogenic calcites could not be explained merely by their higher growth rate but would require either higher surface enrichment than the abiogenic calcites or precipitation from a fluid whose Sr/Ca ratio was about twice that of seawater. The temperature effects observed in coccolith Sr/Ca also cannot reflect surface enrichment phenomena and are likely due to thermodynamic control of Sr partitioning in coccoliths. Surprisingly, although different crystal faces have been shown to produce different levels of surface enrichment (Reeder and Paquette 1995), there was no significant difference in Sr/Ca ratios of species which produce calcite with radially-oriented c-axes ("R units" of Young et al. 1999) from those which have calcite with a combination of radially and vertically-oriented c axes ("V/R units" of Young et al. 1999; Fig. 3b). However, faster crystal growth rates diminish the differences between partitioning on different crystal growth faces (J. Paquette pers. comm. 2002). Coccolith crystal growth rates may be too rapid to express different growth preferences on different crystallographic faces.

Alternatively, biological, rather than crystal kinetic factors may exert the dominant control over variations in coccolith Sr/Ca ratios. If the coccolithophorid cell behaves as a relatively closed system with respect to calcification, using nearly all the Ca and Sr taken up by the cell during calcite precipitation, then cellular control of variations in these ion fluxes might be the dominant control over variations in the coccolith Sr/Ca ratio. Although early culture work with *E. huxleyi* indicated that enrichment of the media in Sr promoted attachment of coccoliths to the cells (Sikes and Wilbur 1980), to date no studies have demonstrated a specific biological role for Sr.

Rickaby et al. (2002) suggest that there may be a rate-dependent discrimination between the biological transport of Sr^{2+} and Ca^{2+} associated with either passive transport through ion channels or active pumping via carrier proteins. This model invokes a selectivity against Sr in uptake. In exponentially growing cells, bicarbonate (HCO_3^-) may enter via a $\text{Ca}^{2+}/\text{HCO}_3^-$ symport (Brownlee et al. 1994). If HCO_3^- pumping were enhanced at faster growth rates as an additional source of C for photosynthesis (as implied by some data showing higher calcification per cell at higher growth rates), then increased HCO_3^- would entail an increased rate of supply of Ca through the ion pumps. If there were a selectivity against Sr in this symport which were reduced at higher pumping rates, the Sr/Ca of the calcifying fluid could increase and produce coccoliths with higher Sr/Ca ratios.

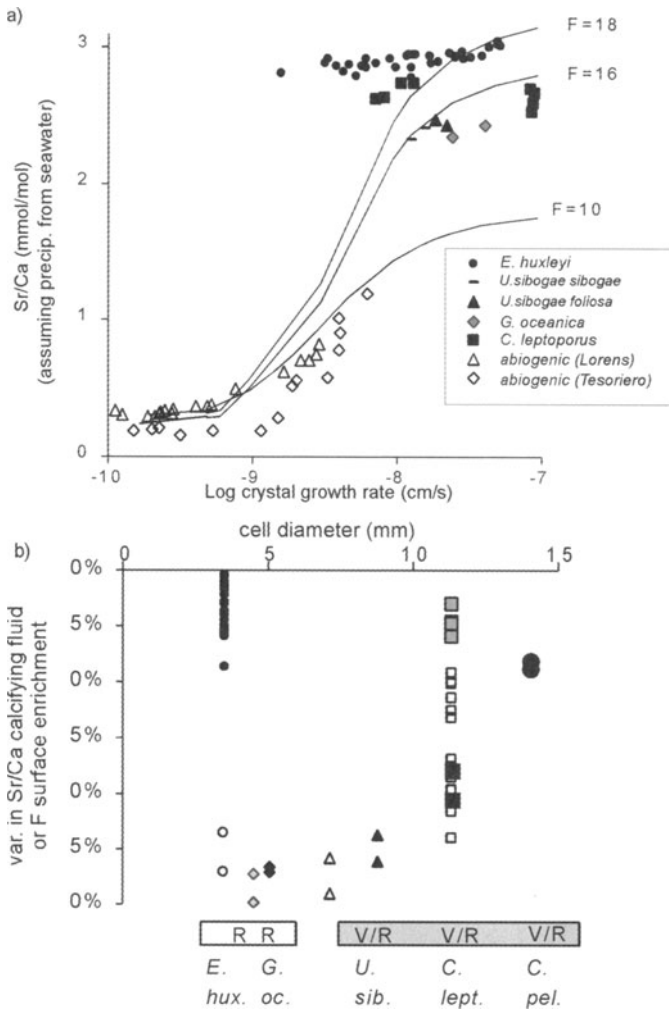


Fig. 3. a) Comparison of coccolith Sr/Ca variations as a function of coccolith crystal growth rate with the surface enrichment model of Watson and Liang (1995). Culture Sr/Ca data for several species are shown (solid symbols) along with data from abiogenic calcite precipitation experiments (open symbols, data from Lorens 1981; Tesoriero and Pankow 1996). Model results for Sr/Ca variation with crystal growth rate (solid lines) are shown for cases where the surface layer of the crystal is enriched 10x, 16x, and 18x ($F=10$, $F=16$, $F=18$) in Sr with respect to equilibrium Sr partitioning in the crystal lattice. Model results and abiogenic calcite results are adjusted to represent precipitation from a solution with the Sr/Ca ratio of seawater, 8.55 mmol/mol. Because the main crystal growth phase may occupy a short part of the time required to produce each coccolith, plotted linear crystal growth rates for coccoliths are minimum rates. b) Sr/Ca ratios in different species compared with crystallographic orientation of the c axis (r =radial units, v/r = combination of radially and vertically oriented units). All data are for cultures at 18°C and all except the smaller circles for *E. huxleyi* are from batch cultures. Data from Stoll et al. 2002b.

The discrepancies in Sr/Ca growth rate responses among surface sediments and different culture experiments suggest that variations in growth rates themselves are not the ultimate control over coccolith Sr/Ca. Consequently, the actual mechanism of Sr/Ca variations in coccoliths must be more complex than the model proposed by Rickaby et al. (2002). However, the carrier protein or ion pumping mechanisms is likely the most useful conceptual framework for further study to understand the mechanisms of Sr variation in coccoliths and their relationships with other biochemical cycles in the cell (see Brownlee and Taylor this volume). Existing data suggests the factor controlling coccolith Sr/Ca is highly correlated with growth rates and productivity in the ocean and, in some culture environments where nutrient-regulated growth rate changes, might be affecting biochemical cycles in the cell.

Prospects for using coccolith Sr/Ca as a paleoproductivity indicator

As for many paleoceanographic proxies, incomplete understanding of the mechanism of Sr/Ca variation need not preclude exploitation of empirical relationships between coccolith Sr/Ca and coccolithophorid productivity. The combination of field and culture data suggests that nutrient-stimulated, rather than light-stimulated changes in growth rate produce the most significant variations in coccolith Sr/Ca. In Holocene sediments from the Mediterranean nutrient-stimulated productivity increases inferred from barite accumulation rates and accumulation rates of organic carbon in anoxic basins are faithfully reproduced by increased Sr/Ca ratios in *E. huxleyi* (Stoll et al. unpublished data). To date the relationship between coccolith Sr/Ca and coccolithophorid productivity is qualitative, rather than quantitative. Quantitative calibrations may be possible for modern coccolith species but will require better techniques for measuring the productivity of specific live coccolithophorid species in the ocean.

Coccolith Sr/Ca ratios offer several advantages which extend the capabilities of current productivity indicators. Many productivity indicators are based on the mass accumulation rate of biogenic components in sediments (opal, organic carbon, carbonate), but mass accumulation rates can be biased by sediment focusing and winnowing, are affected by variable preservation of biogenic materials, and are difficult to measure accurately in pre-Quaternary sediments. Because coccolith Sr/Ca is measured in a single phase, its application is not reliant on determination of mass accumulation rates.

Because coccolithophores in most cases are not the dominant organic carbon producers in the modern ocean, coccolithophorid productivity may not be representative of total productivity in all settings. However, the transfer efficiency of organic carbon from the photic zone to the deep ocean is controlled by the rate of calcium carbonate (Francois et al. 2002). Since coccolithophores are the major producers of calcium carbonate in the open ocean, changes in their productivity may significantly impact the organic C export and carbon burial rates.

The temperature dependence of coccolith Sr/Ca is unlikely to significantly hamper productivity determinations from coccolith Sr/Ca. Since the temperature effect is most likely thermodynamically controlled and the same in all coccolith

species in culture and in the field, most studies have used an independent paleo-temperature indicator (like alkenone undersaturation, oxygen isotopes, or Mg/Ca ratios) to constrain the temperature contribution to Sr/Ca variations (e.g. Stoll and Bains 2003).

If coccolith Sr/Ca were to respond to variations in growth rate in the same way as carbon isotopic fractionation in coccolith organic matter, the coccolith Sr/Ca ratio might improve alkenone carbon isotopic $\epsilon_{\text{alkenone}}$ estimates of paleo- CO_2 (aq) concentrations in the photic zone. In culture studies of *E. huxleyi*, Stoll et al. (2002a) compared the Sr/Ca ratio of coccoliths with the relationship between carbon isotope fractionation (ϵ_p) and the aqueous CO_2 concentration (this latter relationship is frequently expressed as the "b" slope ($(25 - \epsilon_p)/\text{Ce}$); Fig. 4). Overall, the data show only a modest correlation between coccolith Sr/Ca and the "b" slope ($r^2 = 0.31$). For some experiments, correlation was higher and some of the offsets may be explained by variable cell size under different experimental conditions. Nonetheless, the fact that overall correlation is modest indicates that coccolith Sr/Ca does not respond to changing cell physiology in exactly the same way as carbon isotope fractionation in coccolith organic matter, at least under light-limited culture conditions. Slightly better overall correlation was found between coccolith Sr/Ca and the "b" slope in the Equatorial Pacific field study (Stoll and Schrag 2000) and in nutrient-limited culture experiments (Rickaby et al. 2002).

In reconstructing longer term productivity variations ($>10^6$ years) from coccolith Sr/Ca ratios, potential variation in the seawater Sr/Ca ratio must also be considered. The relatively long residence times of Sr and Ca in seawater (millions of years) preclude changes in Sr/Ca ratios of more than a few percent on shorter time-scales (Stoll and Schrag 1998; Stoll et al. 1999). However, changes in sea level or mineralogy of the dominant marine carbonate producers may alter Sr/Ca ratios by tens of percents on longer time-scales (Stoll and Schrag 2001). Modeling likely changes in seawater Sr/Ca ratios from independent records of sea level and carbonate production, or monitoring Sr/Ca ratios in another biogenic carbonate with more constant Sr partitioning (e.g. Lear et al. 2003), may be the best way to deconvolve these multiple influences on coccolith Sr/Ca.

Particularly in Neogene sediments where foraminifera are abundant, the presence of foraminiferal fragments in measured coccolith fractions must be minimized. Since foraminifera have much less Sr than coccoliths, the Sr/Ca ratio of "fine fractions" can be sensitive to changes in the proportion of foraminiferal to coccolith carbonate (see Baumann et al. this volume), as well as changes in the Sr/Ca ratio of either coccoliths or foraminifera (Stoll and Schrag 2000).

Mg/Ca ratios in coccolith calcite

Mg incorporation in calcite is strongly dependent on temperature, and the Mg/Ca of foraminifera is increasingly used for paleothermometry following initial work by Nuernberg et al. (1996). It is likely that coccolith Mg/Ca ratios also reflect their

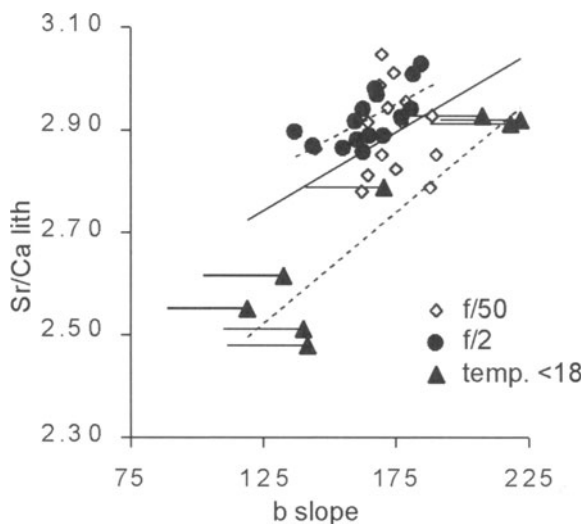


Fig. 4. Sr/Ca in coccolith calcite vs. "b" slope of carbon isotopic fractionation in particulate organic carbon ("b" slope = $25 - (\epsilon_p) * CO_2(aq)$) in *E. huxleyi* continuous cultures. Regression lines are given for all data (solid line, equation $y = 0.0034x + 2.30$; $r^2 = 0.31$), for data at 18°C, f/2 (upper dashed line, equation $y = 0.0026x + 2.49$; $r^2 = 0.47$) and for data at temperatures <18°C (lower dashed line, equation $y = 0.0026x + 2.49$; $r^2 = 0.87$). Horizontal error bars on lower temperature data indicate possible shifts in the b slope due to underestimates of cell size, assuming cells at cooler temperatures are 15% larger than at higher temperatures. Data from Stoll et al. 2002a.

calcification temperature. Estimates of the growth temperature of coccolithophores are important for interpreting productivity variations from coccolith Sr/Ca and for inferring pCO_2 from carbon isotope fractionation in alkenone biomarkers. A Mg/Ca coccolith temperature indicator might complement the alkenone undersaturation proxy and elucidate some of the potential ecological or physiological influence on the latter (e.g. Conte et al. 1998; Epstein et al. 1998) and provide an alternative to foraminiferal Mg/Ca in areas where foraminifera are not present or where the foraminiferal Mg/Ca record might be compromised by selective dissolution due to heterogeneous distribution of Mg in foraminifera.

Early studies of the Mg composition of coccoliths concluded that they were "low-Mg calcite" (<4% $MgCO_3$; Siesser 1977). Recent precise measurements show that coccolith Mg/Ca ratios are extremely low, (0.1 to 0.2 mmol/mol or <0.01% $MgCO_3$), one to two orders of magnitude lower than in foraminiferal calcite (Stoll et al. 2001). These low ratios complicate determination of coccolith Mg/Ca variations, because analytical measurement is difficult for small samples and because cleaning procedures to remove the Mg-rich organic phases and other noncarbonate phases must be extremely efficient. Typical *E. huxleyi* samples from

culture experiments contain $5\text{--}25 \times 10^{-14}$ g Mg/cell in organic fractions, 100 to 500 times higher than that of the CaCO_3 , which contains only 5×10^{-16} g Mg cell⁻¹ (Rosenenthal pers. comm.).

Measurements of Mg/Ca in coccoliths from several species grown in culture suggest that temperature may be an important control on Mg partitioning in coccolith calcite (Fig. 5), but further studies are needed to confirm this result (Stoll et al. 2001). Because coccoliths are much smaller and have a much lower Mg content (compared to foraminifera), the potential advantages of a coccolith Mg/Ca paleotemperature proxy may be outweighed by the greater complexity of cleaning issues. Alkenone undersaturation indices (U_{37}^K) may provide the most reliable index of temperatures at which coccolithophores are growing.

Indicators from stable isotopes in coccolith calcite

Stable isotopic measurements in biogenic carbonates have been a key tool for paleoceanographic studies for nearly half a century, beginning with the pioneering work of Emiliani (1954). The oxygen isotopic ratio is widely used to reconstruct the temperature of ancient oceans and to trace changes in the oxygen isotope ratio of seawater which varies with the evaporation/precipitation balance and with global ice volume. The carbon isotope ratio of biogenic carbonates is used to reconstruct variations in the carbon isotopic composition of dissolved inorganic carbon (DIC) in the ocean, tracking changes in marine productivity and in the carbon cycle. In coccolith carbonate, stable isotope records present special challenges, because the small size of coccoliths does not readily permit separation of the monospecific samples commonly analyzed for other types of carbonates. However, stable isotope records in coccoliths may potentially provide unique paleoceanographic information that cannot be gleaned from studies of other organisms.

For all marine carbonates, the oxygen isotopic ratio depends on the temperature at which the carbonate precipitates as well as the isotopic composition of the seawater from which it forms. Temperature determines the magnitude of the large isotopic fractionation between oxygen isotopes in water and oxygen in CaCO_3 , as predicted theoretically by Urey (1947). During precipitation of CaCO_3 , the heavy ^{18}O is preferentially incorporated into the carbonate while light ^{16}O preferentially remains in the water. This preference is greater at colder temperatures so carbonates precipitated at colder temperatures have higher ratios of $^{18}\text{O}/^{16}\text{O}$ than those formed at warmer temperatures. In the ocean, the oxygen isotopic composition of seawater varies both spatially and temporally. Because oxygen isotopes are strongly fractionated during evaporation (and condensation; Dansgaard 1961), when the heavy ^{18}O preferentially remains in the liquid water, different rates of evaporation in different parts of the ocean lead to spatial gradients of nearly 2 permil in the $^{18}\text{O}/^{16}\text{O}$ of surface seawater which are positively correlated with salinity (Ostlund et al. 1987). The mean oxygen isotopic ratio of seawater is controlled by the hydrological cycle. Snow falling at high latitudes (and very low

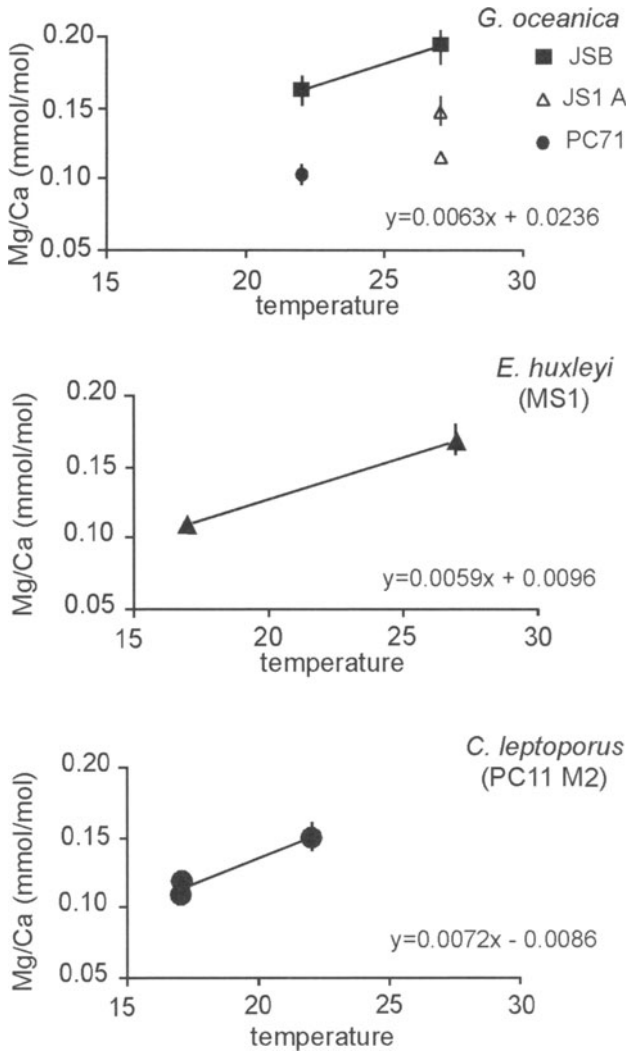


Fig. 5. Mg/Ca ratios in different species of coccoliths from culture after cleaning with bleach/peroxide method. The strain cultured is given in parentheses for *Calcidiscus leptoporus* and *Emiliania huxleyi* and in the legend for *Gephyrocapsa oceanica*. Error bars shown are $\pm 5\%$ estimated uncertainty from possibly incomplete removal of organic phases. Analytical uncertainty is estimated at $<1\%$ r.s.d. From Stoll et al. 2001.

temperatures) has a very low $^{18}\text{O}/^{16}\text{O}$ ratio. When this precipitation is sequestered in growing ice sheets, there is an increase in the average $^{18}\text{O}/^{16}\text{O}$ ratio of the sea-water remaining in the ocean. Pleistocene glacial cycles are recorded by higher $^{18}\text{O}/^{16}\text{O}$ ratios in marine carbonates both because ocean temperatures are colder

(greater fractionation between carbonate and water) and because the $^{18}\text{O}/^{16}\text{O}$ ratio of seawater is higher (large ice sheets).

The carbon isotopic composition of marine biogenic carbonates largely reflects the carbon isotopic composition of dissolved organic carbon in the ocean, since the temperature effect on carbon isotopic fractionation is very small. Within the ocean the spatial gradients in the carbon isotopic composition of DIC are controlled by biological productivity through the removal of isotopically light carbon in organic matter. The relative importance of different inputs of carbon to the ocean-atmosphere system (weathering of organic and inorganic carbon from rocks, release of mantle carbon through volcanic CO_2 , and release of methane hydrate) and relative removal rates of organic and inorganic carbon from the ocean, set the average carbon isotopic composition of DIC in the ocean. The carbon isotopic composition of marine carbonates can be used to reconstruct spatial gradients in the isotopic composition of dissolved inorganic carbon to identify past changes in productivity and ocean circulation (e.g. Broecker 1971), while changes in the global average isotopic composition can be used to infer changes in the carbon cycle (e.g. Dickens 2001).

Empirically-determined equations can be used to calculate the equilibrium isotopic composition of carbonate which forms from a water of known oxygen and carbon isotopic composition at a given temperature. However, many modern biological carbonates do not have the expected equilibrium isotopic compositions predicted from the isotopic composition of the waters in which they formed. These offsets from equilibrium, termed "vital effects", have generally hampered paleoceanographic application of stable isotopes in some organisms, especially coccolithophores. Most paleoceanographic studies have circumvented the vital effects problem by isolating carbonate from a single species and assuming that the vital effect in a given species is constant and does not vary through time. The relative changes in isotopic values can then be interpreted as changes in temperature or in the isotopic composition of seawater.

In Quaternary paleoceanography, this approach is widely applied to foraminifera but not coccoliths whose small size (2–12 μm) precludes "picking" individual specimens to obtain monospecific populations for analysis. In Early Cenozoic and Mesozoic sediments where foraminifera are less common and core material is limited and occasionally indurated, coccolith-dominated bulk carbonate has been a standard phase for stable isotopic analysis (e.g. Bains et al. 1999). Concern over the reliability of these polyspecific bulk carbonate records remains, in part because cultures of coccolithophores show an especially wide range of vital effects in oxygen isotopes (Dudley et al. 1986). Consequently, changes in the relative carbonate contribution of different species in sediments may cause significant changes in the isotopic ratios of the assemblage, potentially obscuring the signals of oceanic variability.

Several studies (summarized in Table 2) have shown that the isotopic variations of Pleistocene polyspecific coccolith-dominated sediment fine fractions generally covary with those measured in planktic foraminifera, although in most records the coccolith fraction isotopes are offset from equilibrium values, either to higher or lower oxygen isotopic compositions. However, in one record from the Caribbean,

the amplitude of glacial/interglacial oxygen isotopic variations was larger in the coccolith fraction than in the planktic foraminiferal record (Anderson and Steinmetz 1983). This discrepancy could not be attributed to changing nannofossil assemblages since *Gephyrocapsa* was the dominant coccolith carbonate contributor throughout. The authors attributed to discrepancy to attenuation of the planktic foraminiferal signal due to stronger dissolution of the planktic foraminifera during interglacials. However, variation of the magnitude of the coccolith vital effect during Pleistocene environmental variations could also have caused or contributed to this discrepancy.

Table 2. Summary of studies of coccolith stable isotopes in sediments.

Study	Size fraction	Age	Location	Result
Anderson and Cole 1975	<44 μm	Pleistocene	Caribbean	$\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ of coccolith fraction covaries with that of planktic foraminifera but offset
Margolis et al. 1975	<44 μm	Cenozoic	Southern Ocean	$\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ of coccolith fraction covaries with that of planktic foraminifera
Goodney et al. 1980	<44 μm	Recent (core top)	Global array	$\delta^{18}\text{O}$ of coccolith fraction not in equilibrium with surface waters but shows temperature dependence
Anderson and Steinmetz 1981, 1983	3–25 μm	Quaternary	Caribbean	$\delta^{18}\text{O}$ of coccolith fraction covaries with that of planktic foraminifera but with larger amplitude (attributed to dissolution biases in foram record). $\delta^{18}\text{O}$ offset of +1 to 2 permil in <i>G. oceanica</i> -dominated coccolith fraction consistent with <i>G. oceanica</i> offset in culture.
Paull and Thierstein 1987	<38 μm , in subfractions	Core top and Last Glacial Maximum	Global array	Confirmed range of non-equilibrium carbon and oxygen isotope effects in different coccolith size fractions in sediments from core tops.
Dudley and Nelson 1989	<38 μm	Quaternary	Tasman Sea	Species-specific vital effects from culture can correct for changing coccolith assemblages, but correction is mostly a constant offset
Stoll and Ziveri 2002	<12 μm , in subfractions	Recent (core top)	Atlantic, Indian, Pacific	$\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ offsets among different species are relatively constant in different sites and in agreement with cultures

Comparable results from foraminiferal and coccolith fine fraction isotope measurements are encouraging for the prospect of using coccolith stable isotopes for traditional paleoceanographic applications in cores where changes in nannofossil assemblages are relatively minor. However, this approach may not be as ideal over major paleoceanographic events where there is significant change in the nannofossil assemblage, like the Cretaceous/Tertiary boundary or terminal Paleocene Event (Bralower 2002). The most reliable paleoceanographic application of coccolith stable isotopes for records of past variations in temperature and the isotopic ratio of seawater is thus dependent on:

1. Establishing the range of carbon and oxygen isotope vital effects in modern coccolithophorid species by growing them in laboratory culture, and establishing whether the vital effects for a given species are constant under variable environmental conditions. The development of a constant "species-specific correction factor" for each species might allow calculation of equilibrium isotopic composition in sediments if the changing nannofossil assemblages were quantified (e.g. Dudley and Goodney 1989).
2. For older sediments where it is not possible to culture extinct species, establish relationships in modern culture studies that allow for some prediction of species-specific vital effects in extinct species, or develop methods to separate monospecific fractions from sediments.

Culture study of stable isotopic vital effects in modern coccolithophores

A large range of oxygen isotope vital effects in coccoliths of different species was first identified in cultures of coccolithophores by Dudley and Goodney (1979) and Dudley et al. (1980, 1986) (Table 3). Culture of eight different species of coccolithophores at different temperatures showed that for a given temperature, some species had oxygen isotopic ratios which were higher (isotopically "heavier") than the equilibrium temperature relation predicted for calcite, while others had oxygen isotopic ratios lower than equilibrium (isotopically "lighter"; Fig. 6). Replicate batch and continuous (chemostat) cultures of the same species in different laboratories generally yielded comparable results suggesting that these vital effects were not artifacts of the culturing technique but species-specific "vital effects". Similar ranges of "vital effects" are hypothesized for planktic foraminifera but the exact magnitudes of foraminiferal vital effects for individual species have been harder to constrain because changing depth habitats may contribute to apparent disequilibrium in the open ocean and only a few species of foraminifera have been cultured in the laboratory for stable isotope analysis (e.g. Bijma et al. 1991).

Dudley et al. (1986) calculated the paleotemperature equations for each of these cultured coccolithophorid species (Fig. 6). For oxygen isotopes, this study provided "species-specific correction factors" for the major coccoliths in recent sediments which allowed Dudley and Goodney (1989) to correct polyspecific coccolith oxygen isotopic records to equilibrium isotopic variations. It did not provide similar factors for carbon isotopes or test whether the species-specific factors were constant.

Table 3. Species-specific offsets from equilibrium (vital effects) in permil at 17°C. Equilibrium oxygen isotopic composition calculated from equation of O'Neil et al. (1969). For carbon isotopes, difference is with respect to calculated equilibrium between calcite and bicarbonate, which is 0.23 permil at 17°C. For *Coccolithus pelagicus*, both the heterococcolith (HET) and holococcolith (HOL) stage coccoliths were measured (for discussion of heterococcolith and holococcolith production, see Billard and Inouye, this volume).

Species	Oxygen isotope offset	Carbon isotope offset	Study
<i>Algirosphaera robusta</i>	2.2		Ziveri et al. 2003
<i>Calcidiscus leptoporus</i>	-2.4		Dudley et al. 1986
<i>Calcidiscus leptoporus</i>	-0.2	-1.6	Ziveri et al. 2003
<i>Coccolithus pelagicus pel. (HET)</i>	1.7	-2.1	Ziveri et al. 2003
<i>Coccolithus pelagicus hyal. (HOL)</i>	-1.1		Ziveri et al. 2003
<i>Emiliana huxleyi</i>	2.8		Dudley et al. 1986
<i>Emiliana huxleyi</i>	3.1	3.2	Ziveri et al. 2003
<i>Gephyrocapsa oceanica</i>	2.0		Dudley et al. 1986
<i>Gephyrocapsa oceanica</i>	2.5		Ziveri et al. 2003
<i>Helicosphaera carteri</i>	-0.1		Ziveri et al. 2003
<i>Oolithotus fragilis</i>	-0.9		Ziveri et al. 2003
<i>Pleurochrysis carterae</i>	-1.5		Dudley et al. 1986
<i>Reticulofenestra sessilis</i>	2.0		Dudley et al. 1986
<i>Syracosphaera pulchra</i>	-2.2		Dudley et al. 1986
<i>Umbilicosphaera hulburtiana</i>	-1.9		Dudley et al. 1986
<i>Umbilicosphaera sibogae</i>	-1.5		Dudley et al. 1986
<i>Umbilicosphaera sibogae foliosa</i>	-0.5	-1.7	Ziveri et al. 2003
<i>Umbilicosphaera sibogae sibogae</i>	-0.3	-1.9	Ziveri et al. 2003

Recent culture experiments of eight species of coccolithophores (as well as heterococcoliths and holococcoliths from different life stages) under identical environmental conditions (nutrients, temperature, light) confirm the large range of interspecific oxygen isotope vital effects and reveal a comparably large range in carbon isotopic vital effects. Oxygen isotopes showed a 5 permil range in epsilon $\delta^{18}\text{O}$ (~ 5 permil range in $\delta^{18}\text{O}_{\text{calcite}} - \delta^{18}\text{O}_{\text{media}}$), with values both above and below the expected equilibrium $\delta^{18}\text{O}$ for precipitation from seawater at 17°C.

Carbon isotopes also showed a 5 permil range in $\epsilon^{13}\text{C}$ ($\sim \delta^{13}\text{C}_{\text{calcite}} - \delta^{13}\text{C}_{\text{media DIC}}$). $\epsilon^{13}\text{C}$ values fall both above and below the expected equilibrium $\epsilon^{13}\text{C}$ for precipitation from seawater bicarbonate at 17°C. This array of species shows a more continuous distribution of oxygen isotopic compositions than in the Dudley et al. (1986) study in which coccolith oxygen isotopic fractionation fell into two distinct groups. The species-specific offsets from equilibrium from all culture experiments are summarized in Table 3.

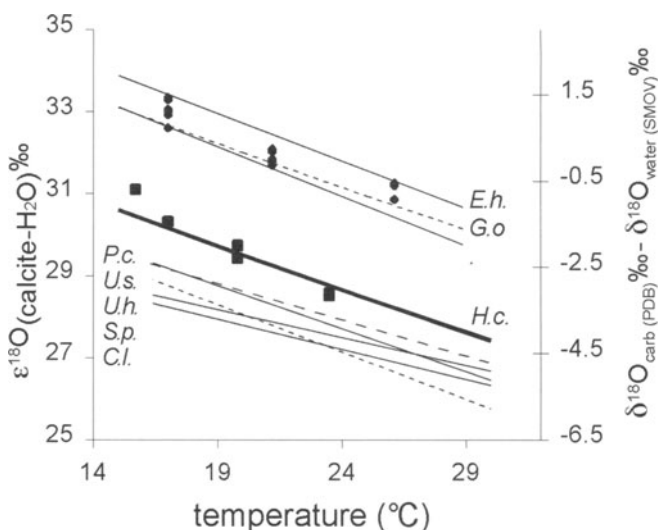


Fig. 6. Disequilibrium factor $\epsilon^{18}\text{O}$ vs. temperature for coccoliths grown in culture. (E. h. = *Emiliania huxleyi*; G. o. = *Gephyrocapsa oceanica*; P. c. = *Pleurochrysis carterae*; U. s. = *Umbilicosphaera sibogae*; U. h. = *Umbilicosphaera hultburtiana*; S. p. = *Syracosphaera pulchra*; C. l. = *Calcidiscus leptoporus*) All results from Dudley et al. (1986) except *G. oceanica* (diamonds) and *H. carteri* (squares) from Ziveri et al. 2003. Bold black line shows equilibrium paleothermometry relation of O'Neil et al. (1969): $1000 \cdot \ln \epsilon = (2.78 \cdot 10^6 \cdot T^{-1}) - 2.89$, where temperature (T) is in degree Kelvin.

These interspecific differences in stable isotope fractionation do not appear to correlate with major phylogenetic divisions of the coccolithophores or biogeography but did correlate with several physiological variables from the culture experiments. The interspecific differences in $\epsilon^{18}\text{O}$ were not correlated to different rates of calcite precipitation in different species, nor to different rates of organic carbon fixation in different species. Interspecific differences were highly correlated with different maximum growth rates of the different species under light saturated and nutrient replete conditions and were moderately correlated with the size of the cell and the cell's surface area/volume ratio (Fig. 7).

Carbon isotopic fractionation was not significantly correlated to different rates of organic carbon fixation in different species nor to different rates of calcite precipitation in different species but was strongly correlated with both the different maximum growth rates of the different species and the cell size and surface area/volume ratio (Fig. 8). However, at growth rates slower than 1 per day, there was no further depletion in $\epsilon^{13}\text{C}$. The vital effects in carbon and oxygen isotopes were moderately correlated with each other but also show a break point where

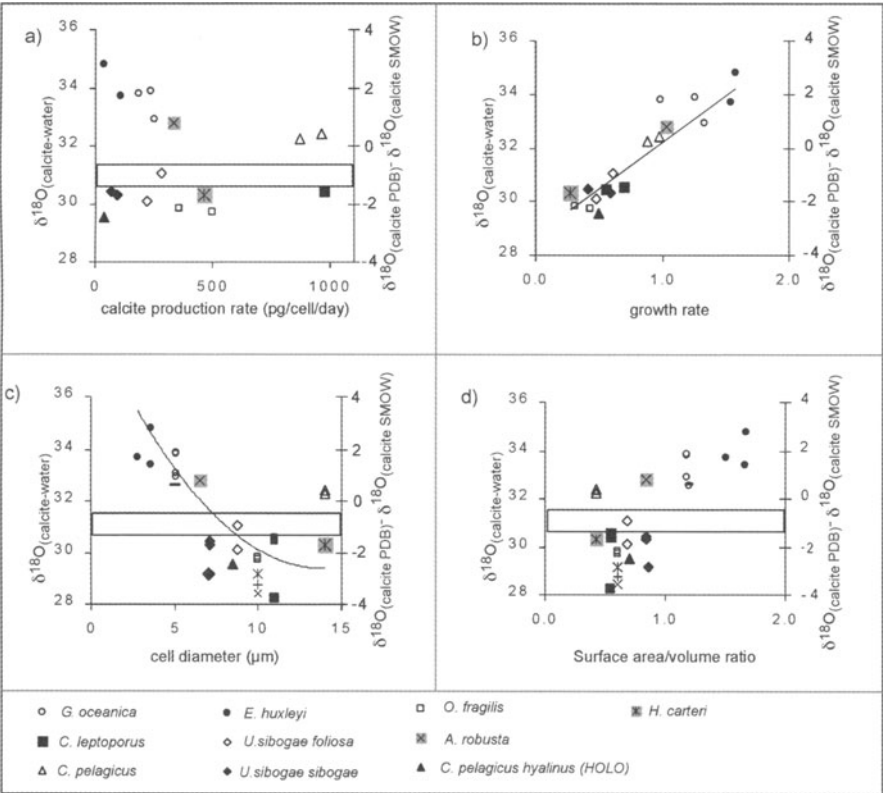


Fig. 7. Oxygen isotopic fractionation in coccolith calcite at 17°C. **a)** Oxygen isotope fractionation vs. calcification rate. **b)** Oxygen isotopic fractionation vs. cell division rate (day^{-1}). The solid line is the least squares fit. **c)** Oxygen isotopic fractionation vs. cell diameter. Polynomial fit excludes data from *C. pelagicus*. **d)** Oxygen isotopic fractionation vs. cell surface area/volume ratio. Data from Ziveri et al. 2003 (a-d); data from Dudley et al. (1986) included in c) and d). Additional species from Dudley et al. (1986) are: *Syracosphaera pulchra* (x), *Umbilicosphaera hultburiana* (+), *Reticulofenestra sessilis* (•). The open bar indicates estimated equilibrium fractionation between carbonate and water at 17°C.

oxygen isotope ratios continue to vary but carbon isotope ratios are relatively constant (Fig. 8d).

If the strong relationship between oxygen and carbon isotope fractionation and cell growth rates observed among different species were also operative at different growth rates in a single species, then the “species-specific vital effects” might not be constant. However, further experiments, using different light intensity to change the growth rates of *Calcidiscus leptoporus* and *Gephyrocapsa oceanica* at constant temperature showed that a comparable growth rate effect is not operative

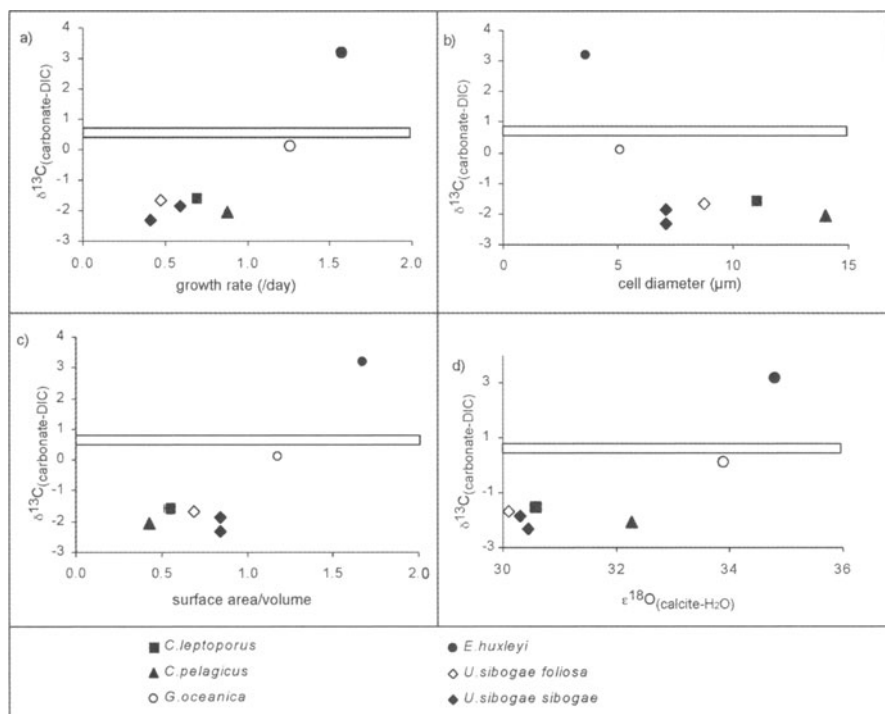


Fig. 8. Carbon isotopic fractionation in coccoliths at of 17°C. **a)** Carbon isotopic fractionation vs. cell division rate (day^{-1}). **b)** Carbon isotopic composition vs. cell diameter. **c)** Carbon isotopic fractionation vs. cell surface area/volume ratio. The isotopic composition of the coccoliths are reported as $\epsilon^{13}\text{C}$ ($\sim \delta^{13}\text{C}_{\text{calcite}} - \delta^{13}\text{C}_{\text{media DIC}}$). **d)** Comparison of coccolith $\epsilon^{18}\text{O}$ and $\epsilon^{13}\text{C}$ data. The open bar indicates estimated equilibrium fractionation between carbonate and water at 17°C. All data from Ziveri et al. 2003.

within a given species (Ziveri et al. 2003). In these experiments, $\epsilon^{18}\text{O}$ values for *G. oceanica* varied by less than 1 permil even as growth rates varied from 1.9–0.5 day^{-1} . (Fig. 9). Likewise, for *C. leptoporus*, decreasing irradiance reduced growth rates from 0.7–0.2 day^{-1} , yet $\epsilon^{18}\text{O}$ varied only 1.5 permil. (Fig. 9). Temperature-induced variations in growth also do not affect $\epsilon^{18}\text{O}$ in these cultures. In cultures of *G. oceanica* growth rate increased from 1.1–2.0 day^{-1} as temperatures increased from 17° to 30°C, yet the slope of the relationship of $\epsilon^{18}\text{O}$ with temperature is indistinguishable from that measured for equilibrium precipitation (O'Neil et al. 1969). If increasing growth rate caused $\epsilon^{18}\text{O}$ in *G. oceanica* to increase at the same rate it does among different species, the growth rate effect would compensate for the $\epsilon^{18}\text{O}$ decrease with temperature for all except the hottest experiment and there would be no correlation between $\epsilon^{18}\text{O}$ and temperature.

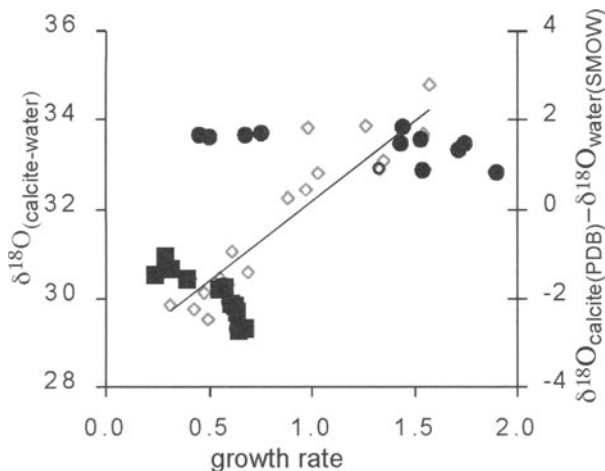


Fig. 9. $\epsilon^{18}\text{O}$ of *G. oceanica* (solid circle) and *C. leptoporus* (solid square) under variable irradiance and growth rate compared with the growth rate effect among different species at constant irradiance as shown in Fig. 7b (open diamonds). All experiments at 17°C.

Why are there stable isotopic vital effects in coccoliths?

Interspecific variations in $\epsilon^{18}\text{O}$ are most highly correlated with growth rate. However, the lack of comparable $\epsilon^{18}\text{O}$ variations with either light or temperature-modulated growth rate variations in a single species suggests that instantaneous growth rate is not the ultimate control over vital effects in coccoliths. The data from multiple growth rates for a single species also indicate that there is nothing unique about the slope of the growth rate vs. $\epsilon^{18}\text{O}$ relationship in species grown at 17°C (Fig. 7b). There is a greater range of growth rates among the small, fastest-growing, species so with reduced irradiance the same variation in $\epsilon^{18}\text{O}$ would occur over a smaller range of growth rates, causing a steeper slope.

These vital effects must reflect some other physiological adaptation correlated with growth rate differences among species. The vital effects were moderately to strongly correlated with cell size or surface area/volume ratio, which varies among species but which would not change significantly (i.e. by more than 20%) in a single species across a broad range of culture conditions. The correlation between cell size and growth rate may reflect cell growth limited by the cell's rate of acquisition of nutrients or C across cell membranes (Popp et al. 1998; Rost and Riebesell this volume), whereby larger cells with lowest surface area/volume ratios may grow more slowly.

The fact that there are consistent relationships between the vital effects and cell size and growth rate across different species suggests that vital effects may arise

from a consistent type of mechanism in all species. If coccolith vital effects resulted from a random array of different processes in different organisms it is unlikely that there would be a systematic, first order relationship with cell size and growth rate. For coccolithophores, which calcify intracellularly in specialized vesicles (see Billard and Inouye this volume), the challenge lies in ascertaining how kinetic and thermodynamic processes of isotopic fractionation are linked to cellular carbon acquisition and carbonate precipitation. This is a daunting challenge since studies have not conclusively distinguished whether C is taken up only as CO_2 by passive diffusion or also by active transport of CO_2 or HCO_3^- (e.g. McConnaughey et al. 1997; Keller and Morel 1999; Brownlee and Taylor this volume; Rost and Riebesell this volume). In reality, the patterns of stable isotopic variations in coccoliths may provide more constraints for unraveling the cellular C and acquisition methods than vice versa.

Three potential effects: 1) kinetic fractionation during calcite precipitation, 2) fractionation of the intracellular C pool during photosynthesis, and 3) pH-dependent fractionation, may contribute to the isotopic variations observed in coccoliths.

1) Kinetic effects on isotopic fractionation during carbonate precipitation are the most widely invoked mechanism for vital effects in biogenic carbonates (McConnaughey 1989). If the precipitation of carbonate is faster than the hydroxylation of carbon dioxide ($\text{CO}_2 + \text{OH}^- \rightarrow \text{HCO}_3^-$), then the faster hydroxylation of ^{12}C - and ^{16}O -bearing CO_2 produces covarying depletions in both ^{18}O and ^{13}C of the carbonate compared to equilibrium. The most rapidly precipitated carbonate is isotopically lighter for both oxygen and carbon. Kinetic effects are not likely to be important where carbonic anhydrase catalyzes equilibrium between carbonate species and water. While carbonic anhydrase has been identified in coccolithophores, its location relative to the calcifying reactions may be important (see Brownlee and Taylor this volume). If it is localized at the site of one reaction but not another, kinetic effects may still play a role in coccolith carbonate. However, kinetic vital effects cannot produce carbonates which are isotopically enriched in ^{13}C or ^{18}O with respect to equilibrium calcite, as is observed for coccoliths of some species (Dudley et al. 1986; Ziveri et al. 2003).

2) If there were significant exchange between the carbon pools used for photosynthesis and calcification, the strong kinetic isotopic fractionation during photosynthesis could influence the carbon isotopic composition of coccoliths (McConnaughey et al. 1997). The residual carbon dioxide not fixed through photosynthesis would be isotopically heavier in carbon, since ^{12}C is preferentially fixed in organic carbon. Conversely, respired CO_2 would be isotopically lighter than CO_2 diffused across the cell membrane. Addition of residual C from photosynthesis could explain the $\delta^{13}\text{C}$ values higher than equilibrium calcite, although they could not explain enriched $\delta^{18}\text{O}$ values.

3) For some biogenic carbonates which calcify extracellularly like foraminifera or corals, the different equilibrium fractionations among different carbonate species with respect to water may also give rise to important variations in the isotopic compositions of carbonates (e.g. Zeebe 1999; Adkins et al. 2003). The oxygen isotopic composition of HCO_3^- is heavier than that of CO_3^{2-} and the relative abun-

dance of these species depends on the pH. If the precipitating carbonate uses HCO_3^- and CO_3^{2-} in their relative abundance in the calcifying fluid, and all of the dissolved inorganic carbon is precipitated, then $\epsilon^{18}\text{O}$ is set by the pH. However, to explain the 5 permil range of $\epsilon^{18}\text{O}$ observed among different coccolith species exclusively through this mechanism would require a pH variation of nearly four pH units, unrealistically large. A small pH effect on $\epsilon^{18}\text{O}$ might be superimposed on other sources of isotopic variation, perhaps explaining the higher than equilibrium $\epsilon^{18}\text{O}$ by lower pH in the smaller species.

Integration of these three potential sources of stable isotopic variations in coccolith calcites will require numerical models of stable isotopic fractionation with cell growth similar to those which have been developed to simulate carbon isotope fractionation in algal organic matter (e.g. Keller and Morel 1999). Such models may be able to account for the likely dependence of kinetic fractionation on the surface area/volume ratio which sets the diffusive CO_2 influx/outflux and hence the proportion of available C fixed by the cell, as well as other effects.

Paleoceanographic application of coccolith stable isotopes

The constancy (to within 1‰) of species-specific oxygen isotopic vital effects among multiple strains of the same species isolated from a wide range of oceanographic settings and even over large changes in cell division rates and calcification rates suggests that in downcore records, vital effects of a given species can be assumed to vary by much less than the interspecific range of vital effects. However, some variations in vital effects might still arise from changes in cell biochemistry with changes in seawater pH or nutrients and should be investigated in future studies.

Recent culture results provide the first species-specific correction factors for carbon isotopic vital effects in coccoliths (Ziveri et al. 2003), although further experiments are needed to confirm that these effects are constant for each species. To date, the constancy of carbon vital effects has been examined only for *E. huxleyi*. In light-regulated cultures of *E. huxleyi* spanning an array of growth rates, the carbon isotopic fractionation between coccoliths and DIC varied by less than 2‰ and in most cases by less than 1‰, and variation was not correlated with growth or calcification rate (Rosenthal et al. 1999). Much more variable carbon isotopic fractionation was inferred for *E. huxleyi* coccoliths in another set of light-regulated growth experiments (Rost et al. 2002). However, this variability may in part reflect analytical uncertainties since in the latter experiment coccolith isotopic composition was not measured directly but estimated from measurements of isotopic fractionation in total particulate carbon and particulate organic carbon (Rost et al. 2002).

Confirmation of a large range of interspecific vital effects in both carbon and oxygen isotopes implies that the effects of changing species compositions are significant. For Pleistocene coccolith-dominated bulk or polyspecific coccolith records, the relative carbonate contribution of each species could be balanced by a

species-specific correction factor, as demonstrated by Dudley et al (1989), as long as the contribution of non-coccolith material were also constrained.

The inability to determine species-specific vital effects in extinct species has been the largest challenge to paleoceanographic application of coccolith stable isotopes in older sediments. For example, Bralower (2002) suggested that the $\delta^{13}\text{C}$ shift in coccolith-dominated bulk carbonate at the onset of the Paleocene/Eocene event could be largely a response to a shift in nannoplankton assemblages rather than a shift in surface water $\delta^{13}\text{C}$ as interpreted by Bains et al. (1999). The relationship between coccolith isotopic vital effects and cell size (and cell surface area/volume) may provide a crude basis for estimating the vital effects in extinct species. For many time intervals, whole coccospheres (cell plus its surrounding covering of coccoliths) are preserved, although rare. It would be possible to estimate the cell diameter from measurements on coccospheres. At present, this approach would give only approximate ($\pm 1\%$ for $\epsilon^{18}\text{O}$ and $\pm 0.5\%$ for $\epsilon^{13}\text{C}$) estimates of vital effects due to scatter in the cell size/growth rate relationship. The diameter of coccoliths constrains the minimum cells size, since cells are typically larger than the coccoliths surrounding them. While still crude, over times of large species turnover and large isotopic shifts (K/T boundary, Paleocene/Eocene event and other methane hydrate liberation events) this approach may provide a way to constrain the extent to which changes in bulk or fine fraction $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$ could be attributed to nannoplankton assemblage changes.

New decanting, density stratified columns and microfiltering techniques (Minoletti et al. 2001; Stoll and Ziveri 2002) have permitted separation of more restricted (in cases nearly monospecific) coccolith fractions from sediments. Although the methods are time-consuming, they remove the necessity for correcting isotopic records for changing nannofossil assemblages and may be the best approach for older sediments where species-specific vital effects cannot be determined in culture. The potential to separate monospecific coccolith fractions from sediments also offers, for the first time, the possibility of using the organic and inorganic C phase of the same organism (*G. oceanica*) to calibrate temperature proxies, based on alkenone undersaturation ratios and species-specific $\delta^{18}\text{O}$ of coccolith calcite. Also, if the constancy of carbon isotopic fractionation with growth rate can be confirmed, then estimates of past carbon isotopic fractionation during photosynthesis (ϵ_p or $\epsilon_{\text{alkenone}}$) might be more reliably obtained from the isotopic difference between alkenone biomarkers and the coccoliths of the alkenone producers (*E. huxleyi* and *G. oceanica*) rather than from fractionation between biomarkers and the carbonate of foraminifera as is current practice (e.g. Jasper et al. 1994). Finally, if models show that coccolith stable isotope fractionation constrains mechanisms of carbon acquisition and calcification in coccolithophores, measurements of coccolith stable isotopes in the past might elucidate how these mechanisms have changed in the past.

Concluding remarks

The chemistry of organic biomarkers and inorganic calcite produced by coccolithophores offer the potential to extract information about past environmental and biological conditions, including: sea surface temperatures (alkenone undersaturation U_{37}^K , coccolith oxygen isotopic ratios, and possibly coccolith Mg/Ca), dissolved and atmospheric CO_2 concentrations (carbon isotopic fractionation in biomarkers or $\epsilon_{alkenone}$), coccolithophorid productivity (coccolith Sr/Ca), and carbon cycling within the ocean and between other carbon reservoirs (coccolith carbon isotopes). Combined study of the stable isotopic fractionation of coccoliths in culture may also elucidate mechanisms of carbon acquisition. One of the greatest assets of coccolithophorid-based proxies is the potential synergy of paleoceanographic studies using multiple indicators all derived from the same organism. As is the case for all paleoceanographic proxies, continued calibration studies are required to further improve our understanding of coccolithophorid-based proxy systems and increase confidence in their paleoceanographic application.

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